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#### Granoff et al.

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#### (54) CHIMERIC FACTOR H BINDING PROTEINS (FHBP) AND METHODS OF USE

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## (58) Field of Classification Search

None

See application file for complete search history.

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#### (57) ABSTRACT

Chimeric fHbps that can elicit antibodies that are bactericidal for different fHbp variant strains of *N. meningitidis*, and methods of use, are provided.

#### 18 Claims, 39 Drawing Sheets

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(genome-derived neisseria) antigen 1870), a promising group B meningococcal vaccine candidate" *Clin. Vaccine Immunol.* 13(7):758-763.

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E.a.8

E.α.1

E.α.1

E.α.2

E.α.17

E.α.12

D.α.2 D.α.4 D.α.3 D.α.4 D.α.3 D.α.1 C.a.14 C.a.10 C.a.5 C.a.6 C.a.2 C.a.2 C.α.2 fHbp Variable Segment<sup>1</sup> B.α.1 B.α.1 B.α.1 B.α.7 B.α.1 B.α.1 B.α.1  $\alpha$ A.α.13 A.α.12 A.α.2 A.α.2 A.a.3 A.α.3 A.a.1 Fig. 1A: Table 7, Source strains and characteristics of unique factor H binding protein variants. Element เนเอา-M Ġ G G Ç G G G AASS69 16 AASS69 20 AAS569 17 NP 274866 NP 283399 Acc. No. None\* None\* Genbank Group fHbp Characteristics Modular family Peptide ID C) 3 4 v, 9 Group  $\propto$  $\alpha$  $\mathbf{\alpha}$  $\mathbf{\alpha}$  $\underline{\gamma}$  $\alpha$  $\underline{\alpha}$ ) Jusius V 7,13-1 Region 23,1 4  $\operatorname{Por}$ 5,2 5,2 7,1 V Variable Cpx, 41/4 Clonal 32 Ξ Ξ Ξ 35 4 MLST  $\mathbf{IS}$ 154 35 74 4 Group Capsular  $\propto$  $\mathcal{O}$  $\mathbf{m}$ ₹  $\mathbf{\alpha}$  $\mathfrak{A}$ Gam bia Country C SOS  $\Omega$ CO (Tettelin et al., (Parkhill et al., (Welsch et al., (Welsch et al., Welsch et al., (reference) M2937 M2197 M4105 M6190 Strain 2004) 2000) 2004) 72491 2000)

<sup>1</sup> For each variable segment, distinct sequence variants were assigned a unique identifier beginning with a letter, A through E, to represent the segment; followed by an  $\alpha$  or  $\beta$  to indicate the presence of residues with the respective types described above, followed by a number for each distinct sequence

Modular group see Figure 8.

The mature fHbp begins with a cysteine residue that is lipidated, which is followed by three invariant amino acid residues, SSG. This invariant sequence is followed by a repetitive variable sequence consisting of 1 to 6 glycine and/or serine residues and then by two invariant glycine residues

E.α.16 E.α.10  $E.\alpha.24$  $E.\alpha.23$ E.α.6 E.α.7 D.α. 1 D.a.2  $D.\alpha.6$  $D.\alpha.5$  $D.\alpha.2$ D.a.1 D.α.1 D.α.1 D.a.1 D.a.1 D.α.1 C.α.16 C.α.15 C.α.6 C.a.3 C.α.5 C.α.2 G.α.7 C.α.1 C.α.1 G.α.1 C.α.1 B.α.5 B.α.2 B.α.1 B.α.1 B.α.2 B.α.4 B.α.1 B.a. 1 B.a. 1 B.c. 1 A.α.2 A.α.2 A.α.3 A.α.2 A.α.2 A.α.2 A.α.3 A.α.3 A.α.2 A.α.1 A.α.1 GG GSG G  $\mathfrak{O}$ Ŋ  $\mathcal{D}$  $\mathfrak{O}$  $\overline{\mathbb{C}}$ C Ü C G AAR8448 3 AAR8448 2 ACI31833 ACB3815 4 AAR8448 6 EU337063 ACB3814 AAR8447 4 AAS5691 8 GQ21977 4 GQ21977 5 99 57  $\frac{2}{12}$ 13 4 11 40 10 54 6  $\alpha$ М  $\mathbf{m}$  $\mathbf{g}$  $\mathfrak{L}$  $\mathbf{m}$  $\mathfrak{A}$  $\underline{\alpha}$  $\mathbf{m}$  $\simeq$  $\mathbf{m}$  $\begin{vmatrix} 5- \\ 1,10- \\ 8 \end{vmatrix}$ 7-2,4 19-1,26 22,9 18,2 5,6 17,1 6-3 5,2 5,2 Z 41/4 4 41/4 4 41/4 4 269 NA NA Ä NA =  $\Box$  $\prod$ 1161 136  $^{N}$  $^{NA}$ NA NA 4 =  $\square$  $\prod$ 41 W-135 B  $\mathbf{\alpha}$  $\mathbf{m}$  $\simeq$  $\mathfrak{A}$  $\mathbf{p}$  $\alpha$  $\mathbf{m}$  $\mathbf{m}$  $\mathbf{g}$ New Zeala nd Mali UK UK Sil Si Sil Si (Fletcher et al., 2004) (Fletcher et al., 2004) (Fletcher et al., 2004) (Fletcher et al., (Welsch et al., (Beernink et al., 2009) M05 240018 (Beernink et M01 240185 (Beernink et M01 240993 (Beermink et M97 250571 Mali 29/07 NZ98/254 CDC-983 03S - 0501al., 2006) al., 2008) 03S-0444 CDC-852 al., 2006) 2004) 6940

Fig. 1B: Table 7, Source strains and characteristics of unique factor H binding protein variants.

	c.1 E.α.11	ι.1 Ε.α.22	c.2 Ε.α.1	c.1 E.α.21	τ.1 Ε.α.4	c.3 Ε.α.5	ι.1 Ε.α.20	c.6 E.α.19	ι.1 Ε.α.15	ι.1 Ε.α.13	ι.2 Ε.α.1
	D.α.1	D.α.1	D.α.2	D.α.1	D.α.1	D.α.3	D.α.1	D.α.6	D.α.1	D.α.1	D.α.2
	C.α.19	C.a.1	C.α.18	C.α.17	C.α.1	C.α.9	C.α.3	C.α.12	C.α.13	C.α.3	C.a.7
	B.α.1	Β.α.2	Β.α.1	B.α.1	Β.α.2	Β.α.1	Β.α.6	B.α.1	Β.α.1	Β.α.1	Β.α.1
	<b>A</b> .α.1	Α.α.2	Α.α.9	A.α.10	Α.α.2	Α.α.11	Α.α.7	Α.α.8	Α.α.6	Α.α.2	A.α.3
riants.	Ð	Ð	9	G	9	Ð	66 686 6	Ð	Ð	9	Ð
ng protein va	AAR8448 4	ACB3814	ACB3814 4	ACB3814 5	ACB3814 6	ACB3815	ACH8160 4	ACH8160 5	ACI31835	ACI31836	ACF3543 2
bindi	I	I	I	_	Н	П	П	Н	ш	ы	I
tor H	28	99	61	62	9	99	99	71	73	74	78
ue fac	В	B	В	Ж	m	m	В	В	2	e e	В
f uniq	_	-	-	_	_	-	_	-	-	_	-
istics o	NA	22,1	18,2 5-1	22,9	19- 1,15- 11	non- typa blc	22,9	22,9	5- 1,10-	19,2	5,2
naracter	NA	569	269	569	269	269	269	269	UA	UA	11
s and cl	NA	269	1163	1163	5970	7558	352	275	751	5403	11
strain	В	B	В	8	B	В	В	B	×	×	B
7, Source	NA	UK	UK	UK	UK	Germ any	UK	UK	Burki na Faso	Ugan da	Sn
Fig. 1C: Table 7, Source strains and characteristics of unique factor H binding protein variants.	CDC-1343 (Fletcher et al.,	2004) M05 240116	M05 240172	M06 240003	M06 240006	M06 241344	M00 242724	M00 243040	BuFa 2/97 (Beermink et al., 2009)	Ug 10/06 (Beernink et al., 2009)	03S-0408 (Beermink et

	E.α.18	Ε.α.25	E.α.26	E.α.7	E.β.6	E.β.4	E.β.5	E.β.4	E.β.5	E.β.2
	D.a.3	D.α.3	D.α.9	D.a.1	D.β.1	D.β.2	D.β.2	D.β.3	D.β.2	D.α.1
	C.a.20	C.α.21	C.a.22	C.α.6	C.B.9	C.β.10	C.B.11	C.B.1	C.β.5	C.β.1
	Β.α.4	Β.α.1	Β.α.1	Β.α.1	B.B.1	B.β.1	В.В.1	B.B.1	В.В.1	Β.β.1
	Α.α.14	Α.α.15	Α.α.2	Α.α.16	Α.β.1	A.β.6	Α.β.1	A.ß.1	A.ß.1	A.ß.2
iants.	Ĵ. SG	2 U	9	66 686 6	66 686 6	989 686 6	08 08 08 0	7 PA	SGG	GG GS
g protein var	ABX7255 8	GQ21976 7	GQ21976 8	GQ21 <i>977</i> 0	ABF8202 9	GQ21 <i>977</i> 6	ACA5254	AAR8443 7	ACB3814 8	ACM4493 9
indin	I				п	ш	=	=	п	П
or II k	08	<b>%</b>	68	96	28	29	30	46	2	72
e fact	В	B	g	g	A	A	K	K	A	A
uniqu	1	_	_	_	co	с С	co	m	m	co
istics of	NA	22,1	7,16	22,1 4	23,1	22,1	22,1	non- typa ble	12- 1,13-	19- 1,15- 11
aracter	4821	162	32	213	41/4	162	213	NA	269	269
s and ch	4821	162	32	213	437	5573	213	V Z	492	283
strain	C	B	B	B	В	B	<u>~</u>	<u>~</u>	В	В
, Source	China	Sn	Sn	Sn	Sn	Sn	UK	ns	UK	UK
Fig. 1D: Table 7, Source strains and characteristics of unique factor 11 binding protein variants.	053442 (Peng et al., 2008)	SK80 (Beemink <i>et</i> <i>al.</i> , 2008)	SK84 (Beemink et al., 2008)	SK141 (Beemink <i>et</i> al., 2008)	M1239 (Masignani et al., 2003)	(3S-0669 (Beermink et al., 2006)	M01 240988	CDC-1135 (Fletcher et al., 2004)	M06 240137	M01 242162

	E.β.3	E.β.3	Е.β.1	E.β.1	E.β.1	E.β.1	Ε.α.4	E.α.14	Ε.α.5	E.α.3	Ε.α.4
	D.β.1	D.β.1	D.β.1	D.β.1	D.β.1	D.β.1	D.α.1	D.α.1	D.α.1	D.α.1	D.α.1
	C.β.1	C.α.2	G.β.2	C.B.2	C.β.3	C.β.3	C.α.4	C.α.8	C.α.4	C.α.4	C.α.11
	В.β.1	B.α.1	Β.α.1	Β.α.1	B.α.3	B.α.3	Β.α.1	Β.α.1	Β.α.1	Β.α.1	B.α.1
	A.β.8	<b>A.</b> α.1	Α.α.1	Α.α.2	Α.α.1	Α.α.4	A.β.3	A.β.9	A.β.3	A.β.3	A.β.3
iants.	GG GS	9	99	99	99	G	66 686 6	66 686 6	66 686 6	68 686 6	66 686 6
characteristics of unique factor H binding protein variants	ACJ04735	GQ21977 1	ABY2651 8	ACI31834	ACM4495	AAR8442 7	ABL1423 2	AAR8448 1	ACB3814 9	ACM4494 8	ACM4495
bindin	Π	Ħ	Ħ	Ш	Ш	E	N	VI	IV	IV	VI
tor H	9/	21	22	23	24	25	15	55	59	98	87
uc fac	A	V	V	A	A	<	8	В	В	В	В
f unig	m	2	7	2	2	2	-	_	<u> </u>		<u> </u>
istics of	22- 1,14	22,1 4	5,2	5-1,2	22,1 4	NA	19- 1,15- 11	7-1,1	17,1	7-2,4	21,1
aractei	32	162	11	111	213	NA	269	NA	269	41/4 4	41/4
		162	11	11	3496	NA	283	NA	Z	5861	839
strain	B	В	၁	W- 135	В	8	B B	<b>B</b>	B	В	B
7, Source	Sn	Sn	Sn	Burki na Faso	UK	NA	Sn	Sn	CaN Ada	UK	UK
Fig. 1E: Table 7, Source strains and	03S-0451 (Beemink et al., 2006)	MD1397 (this study)	RM1090 (Beernink et al., 2008)	Bula 2/03 (Beernink et al., 2009)	M08 240117	6275	NM452	CDC-1573 (Fletcher et al., 2004)	M06 241169	M08 240004	M08 240077

E.β.2 E.B.3 E.β.2 E.β.2 E.β.3 E.β.9 E.β.2 E.B.4 E.B.1 D.a.8 D.α.1 C.β.8 C.β.2 C.B.5 C.B.2 C.β.2 C.B.4 C.B.1 C.B.1 C.B.1 C.B.1 C.β.1 B.B.1 B.α.1 B.α.1 B.ß.1 B.ß.1 B.ß.1 B.ß.1 B.α.1 B.β.1 B.ß.1 B.ß.1 A.β.2 A.B.2 A.β.2 A.β.2 A.β.5 A.B.4 A.B.7 A.α.1 A.B.1 A.α.1 A.α.1 ACB3813 GG 66 686 6 GG GSG 66 GSG GSG S SGS GG GG 99 GG Fig. 1F: Table 7, Source strains and characteristics of unique factor H binding protein variants. M01 240355 | UK | B | 213 | 213 | 22,1 | 3 | A | 31 | V | ACB3813 | GG Ç Ġ ٥ AAR8443 6 | AAR8443 | 8 AAR8445 3 ACM4493 8 ACH4823 4 ACM4494 GQ21977 3 ABC5906 1 GQ21977 2 None\* M M VI > 19 45 82 16 29 30 79 82 17 47 V ⋖ V V ₹ ₹ ₹ ≺ < ⋖ 3 d 3 co. 22,1 7-1,1 5-2,10-19-1,15-36 12,1 21,1 6 22,1 4 18,2 5,6 5,2 5,2 41/4 162 213 269 UA ٧Z NA  $\Box$  $\infty$ 6874 2391 162 153 ΥN 569 NA $\Box$ 4  $\alpha$  $\mathbf{m}$ Ç M  $\mathbf{\alpha}$ m 2  $\mathbf{m}$  $\mathbf{x}$ Germ Austr alia Germ any US M any Si (Plested & Granoff, 2008) (Masignani et al., 2003) (Madico et al., MD1475 (this (Fletcher et al. (Fletcher et al M97 252153 M06 240138 M08 240109 M98 250771 (Beernink et 03S-0150 al., 2006) C2120 C4678 S3032 2004) 2004) 2006)

Fig. 1G: Table 7, Source strains and characteristics of unique factor H binding protein variants.	7, Sourc	e strain	s and cl	naracteri	istics of	uniqu	tact	or H1	oindir.	ng protein var	iants.					
M0579	SN	В	43	41/4	5,2	2	A	70	IA	VI None*	99	Α.α.1	Β.α.1 С.β.6		D.α.1	E.β.1
ISS1024	Italy	၁	1860	11	5,-	2	A	27	VI	None*	99	Α.α.2	Β.α.1	C.B.4	D.α.1	E.β.2
M98 250809 (Fletcher et al., 2004)	UK	В	NA	NA	7,16	7	A	49	IA	AAR8445 2	99	Α.α.1	B.α.1	C.β.2	D.α.7	E.β.2
NMB (Fletcher <i>et al.</i> , 2004)	SN	В	NA	8	5- 1,2-2	7	A	50	IA	AAR8445 4	99	Α.α.1	B.α.1	C.β.7	D.α.1	E.β.2
M08 240014	NK	В	275	269	22,9	2	V	89	IV	ACH8160 7	99	Α.α.3	Β.α.2	C.β.2	D.α.1	E.β.1
8047 (Beernink & Granoff, 2008)	Sn	В	∞	8	5- 1,2-2	2	A	11	IV	ACJ45782	99	Α.α.5	B.α.1	C.B.4	D.α.1	E.β.2
M06 241348	Germ any	В	3476	269	10	2	A	83	IN	ACM4493 6	99	Α.α.1	Β.α.1	C.β.2	D.α.1	Е.β.7
M08 240023	NK	В	88/9	41/4	21,1	m	A	28	IA	ACM4494 GG 3 GSG G	l	Α.β.1	B.β.1	C.β.1	D.α.1	E.β.8

g o S

21 TGKLKNDKV TSKLKNDKV TGKLKNDKV TGKLKNDKV TGKLKNDKV TGKLKNDKV GNGDSLN TGKLKNDKI TGKLKNDKV TGKLKNDKV TGKLKNDKV TGKLKNDKV TGKLKNDKV TGKLKNDKV TGKLKNDKV TGKLKNDKV TGKLKNDKI TGKLKNDKV IGKLKNDKV TGKLKNDKV TGKLKNDKV TGKLKNDKV TGKLKNDKV TGKLKNDKI Fig. 2A: Table 8, Segment A. Unique sequences in fHbp variable segment A and number from each variant group GNGDSTN GNGDSIN GNGDSLN GNGDSTN GNGDSTN GNGDSLN GNGDSLN GNGDSLN GNGDSLN GNGDSTN GNGDSTN GNGDSTD GNGDSTN GNGDRLN GNGDSLD GNGDSIN GNGDSTN GNGDSTN GNGDSIN GNGDSLN GNGDNLN GNGDSLN GNGDSTN GNGDSTN GNGDSLN GNGDSLN LAAQGVEKTY QSVRKNEKLK LAAQGAEKTY QSVRKNEKLK LAAQGAEETY QSVRKNEKLK LAAQGAEKTY QSVRKKEKLK LAAQGAEKTY QSVRKNEKLK LAAQGAEKTY QSVRKNEKLK LAAQGAEKTY QSVRKNEKLK LAAQGAEKTY QSVRKNEKLK LAAQGAEKTY LAAQGAEKTY LAAQGAEKTY LAAQGAEKTY QSVRKNEKLK LAAQGAEKTY DALITPLDHK DKSLQSLTLD QSVRKNEKLK LAAQGAEKTY DKSLQSLTLD QSVRKNEKLK LAAQGAEKTY LAAQGAEKTY QSVRKNEKLK LAAQGAEEIY QSVRKNEKLK LAAQGAEKTY LAAQGAEKTY LAAQGAEKTY LAAQGAEKTY LAAQGAEETY QSVRKNEKLK LAAQGAEKTY QSVRKNEKLK LAAQGAEKTY DKGLQSLMLD QSVRKNEKLK LAAQGAEKTY DKGLQSLTLD QSVRKNEKLK LAAQGAEETY QSVRKNEKLK Sequence DKGLQSLTLD DKGLQSLTLD DKGLRSLTLD DKGLRSLTLD DKGLQSLTLD DKGLQSLTLD DKGLQSLTLD DKGLQSLILD DKGLQSLTLD DKGLQSLTLD DKSLQSLTLD DKSLQSLTLD DKSLQSLTLD DKGLQSLALD DKGLQSLTLD DKGLRSLMLD DKGLQSLTLD DKGLQSLTLD DKGLQSLTLD DKGLQSLTLD DKSLQSLTLD DKSLOSLTLD DKGLQSLTLD DALTTPLDHK DALTAPLDHK DALTAPLDHK /AADIGAGLA DALTAPLDHK DALTAPLDHK DALTAPLDHK DALTALPDHK DALTAPLDHK DALTVPLDHK DALTAPLDHK DALTTPLDHK DALTAPLDHK DALTAPLDHK DALTAPFDHK DALTAPLDHK VAADIGVGLA VAADIGAGLA VAADIGAGLA VAADIGAGLA /AADIGTGLA /AADIGAGLA JAADIGAGLA /AADIGARLA VAADIGAGLA VAADIGAGLA VAADIGAVLA VAADIGTGLA VAADIGAGLA VAVDIGTGLA VAADIGTGLA VAADIGAGLA VAADIGAGLA /AADIGAGLA VIADIGAGLA VAADIGAGLA /AADIGAGLA /AADIGTGLA VAADIGAGLA /AADIGAGLA VTADIGTGLA /AADIGAGLA  $A.\alpha.17$ A.α.22 A.α.12 A.a.13 A.α.15 A.α.16 A.α.18 A.a.20 A.a.24 A.a.19 A.a.27 A.a.14 A.a.21 A.a.23 A.α.10 A.α.11 A.α.9 Α.α.8 A.α.2 A.0.3 Α.α.4 A.α.5 A.α.6 A.a.7 ₽

Fig. 2A (Con't): Table 8, Segment A. Unique sequences in fHbp variable segment A and number from each variant group.

A 9 28	WAADTGAGI,A	DAT, TAPI, DHK	DKGLOSLILD	OSVRKNEKLK	LSAQGAEKTY	GNGDSTN	TGKLKNDK	42
A 0.29	VAADIGTGLA	DALTAPLDHK	DKGLQSLTLD		LAAQGAEKTY	GNGDSTN	TGKLKNDKI	43
A.0.30	VAADIGAGLA	DALTAPLDHK	DKGLQSLTLD	QSVRKNEKLK	LSAQGAEKTY	GNGDSLN	IGKLKNDKV	44
A.0.31	VAADIGIGLA	DALTAPLDHK	DKGLOSLMLD	QSVRKNEKLK	LSAQGAEKTY	GNGDSLN	TGKLKNDKI	45
A.α.32	VIADIGIGLA	DALTAPLDHK	DKGLQSLTLD	QSVRKNEKLK	LAAQGAEKTY	GNGDSTN	IGKLKNDKV	46
A. a. 33	VAADIGTGLA	DALTALPDHK	DKGLQSLTLD	QSVRKNEKLK	LAAQGAEKTY	GNGDSTN	TGKLKNDKV	47
A.α.34	VAADIGAGLA	DALTAPLDHK	DKGLQSLTLN	QSVRKKEKLK	LAAQGAEKTY	GNGDSLS	TGKLKNDKV	48
A.α.35	VAADIGAGLA	DALTAPLDHK	DKGLQSLTLD	QSVRRNEKLK	LAAQGAEKTY	GNGDSTN	TGKLKNDKV	49
A.a.36	VAADIGAGLA	DALTAPLDHK	DKSLQSLTLD	QSVRKNEKLK	LAAQGAEKTY	GNGDSIN	TGKLKNDKI	20
A.α.37	VAADIGAGLA	DALTAPLDHK	DKGLQSLMLD	QSVRKNEKLK	LSAQGAEKTY	GNGDSIN	TGKLKNDKV	51
A.a.38	VAADIGTGLA	YALTAPLDHK	DKSLQSLTLD	QSVRKNEKLK	LAAQGAEKTY	GNGDSTN	QTGKLKNDKV	52
A.α.39	VIADIGIGLA	DALTAPLDHK	DKGLQSLMLD	QSVRKNEKLK	LAAQGAEKTY	GNGDSLN	IGKLKNDKI	5
A.α.40	VAADIGAGLA	DALTAPLDHK	DKGLQSLMLD	QSVRKNEKLK	LAAQGAEKTY	GNGDSLN	IGKLKNDKI	54
A.α.41	VAADIGTGLA	DALTAPLDYK	DKGLQSLMLD	QSVRKNEKLK	LSAQGAEKTY	GNGDSTN	IGKLKNDKV	22
A 0.42	VAADTGAGLA	DALTAPLDYK	DKGLOSLTLD	QSVRKNEKLK	LAAQGAEKTY	GNGDSLN	IGKLKNDKV	26
A.0.43	VAADIGAGLA	DALTAPLDHK	DKGLQSLTLD	QSVRKNEKLK		GNGDSTN	TDKLKNDKV	57
A.0.44	VAADIGAGLA	DVLTAPLDHK	DKGLOSLILD	QSVRKNEKLK	LAAQGAEKTY	GNGDSLN	IGKLKNDKV	28
A.0.45	VAADIGAGLA	DALTTPLDHK	DKGLRSLMLD	QSVRKNEKLK	LSAQGAEKTY	GNGDSLN	IGKLKNDKV	59
Αα.46	VVADIGAGLA	DALTAPLDHK	DKGLOSLMLD	QSVRKNEKLK	LAAQGAEKTY	GNGDILN	TGKLKNDKV	09
A 0.47	VAADIGAGLA	DVLTAOLDHK	DKGLQSLTLD	QSVRKNEKLK	LAAQGAEKTF	KAGDKDN	SLNTGKLKNDKI	61
Αα.48	TAADIGAGLA	DALTAPLDHK	DKSLOSLILD	QSVRKNEKLK	LAAQGAEKTY	GNGDSLN	TGKLKNDKV	62
Α.α.49	VAANIGAGLA	DALTAPLDHK	DKSLQSLTLD	QSVRKNEKLK	LAAQGAEKTY	GNGDSLN	TGKLKNDKV	63
A.α.50	VAADIGAGLT	DALTAPLDHK	DKSLQSLTLD	QSVRKNEKLK	LAAQGAEKTY	GNGDSLN	IGKLKNDKV	64
A.α.51	VAADIGAGLA	DALTAPLDHK	DKGLQSLMLD	QSVRKNEKLK	LAAQGAEETY	GNGDSLN	IGKLKNDKV	65
A.α.52	VAADIGAGLA	DALTAPFDHK	DKGLQSLTLD	QSVRKNEKLK	LAAQGAEKTY	GNGDSLN	TGKLKNDKI	99
A.B.1		DALTAPLDHK	DKGLKSLTLE	DSIPQNGTLT	LSAQGAEKTF	KAGDKDNSLN	SLN TGKLKNDKI	67
A.B.2	VAADIGTGLA	DALTTPLDHK	DKGLKSLTLE	DSIPQNGTLT	LSAQGAEKTF	KAGDKDNSLN		89
A.B.3	VAADIGAGLA	DALTAPLDHK	DKGLKSLTLE	DSISONGIFL			-	9 (
A.B.4	VAADIGAGLA	DALTAPLDHK	DKGLKSLTLE	DSISONGIFT		KVGDKDNSLN	•	7.0
A.B.5	VAADIGTGLA	DALTAPLDHK	DKGLKSLTLE	DSISONGILT		KVGDKDNSLN	_	7 6
A.β.6	VAADIGAGLA	DALTAPLDHK	DKGLKSLTLE				-	7 (
A.B.7	VAADIGAGLA	DALTAPLDHK	DKGLKSLTLE	DSIPQNGTLT	LSAQGAEKTF	KAGDKDNSLN	SLN TGKLKNDKI	73

Fig. 2A(Con't): Table 8, Segment A. Unique sequences in fHbp variable segment A and number from each variant group.

74 75	9/	77	78	79	80	81	85	83	84
SLN TGKLKNDKI TGKLKNDKV	TGKLKNDKV	DKGLKSLTLE DSIPQNGTLT LSAQGAEKTF KAGGKDN SLNTGKLKNDKI	DKGLKSLILE DSIPQNGTLI LSAQGAEKIF KVGDKDN SLNIGKLKNDKI	DKGLKSLILE DSIPQNGTLI LSAQGAEKTF KAGDKDN SLNTGKLKNDKI	DKGLKSLILE DSIPQNGILI LSAQGVEKIF KAGDKDN SLNIGKLKNDKI	DKGLKSLTLE DSISQNGTLT LSAQGAERTF KAGNKDN SLNTGKLKNDKI	DKGLKSLILE DSIPQNGTLI LSAQGAEKTF KAGDKDN SLNTGKLKNDKI	DKGLKSLILE DSISQNGTLT LSAQGAERTF KAGDKDN SLNTGKLKNDKV	DKGLKSLILE DSIPQNGTLT LSAQGAEKTF KAGDKDN SLNTGKLKNDKI
KAGDKDN	GNGDSTN	KAGGKDN	KVGDKDN	KAGDKDN	KAGDKDN	KAGNKDN	KAGDKDN	KAGDKDN	KAGDKDN
DKGLKSLTLE DSIPQNGTLT LSAQGAEKTF KAGDKDNSLN DKGLKSLTLE DSISONGTLT LSAOGAEKTY GNGDSLN	NKGLKSLTLE DSIPONGTLT LSAQGAEKTY GNGDSLN	LSAQGAEKTF	LSAQGAEKTF	LSAQGAEKTF	LSAQGVEKTF	LSAQGAERTF	LSAQGAEKTF	LSAQGAERTF	LSAQGAEKTF
DSIPQNGTLT	DSIPQNGILI	DSIPQNGILI	DSIPQNGTLT	DSIPQNGTLT	DSIPQNGTLT	DSISQNGTLT	DSIPQNGTLT	DSISQNGTLT	DSIPONGTLT
DKGLKSLTLE	NKGLKSLTLE	DKGLKSLTLE							
DALTAPLDHK DALTAPLDHK	DALTAPLDHK	DALTAPLDHK	DALTAPLDHK	DALTIPLDHK	DALTAPLDHK	DALTAPLDHK	DALTAPLDHK	DALTAPLDHK	GTGLA DALTAPLDHK
IAADIGTGLA	VAADIGAGLA	VAADIGIGLA	VAADIGTGLA	VAADIGAGLA	VAADIGAGLA	VAADIGAGLA	VTADIGIGLA	VAADIGAGLA	VVADIGTGLA
A.B.8	A.B.10	A.B.11	A.β.12	A.B.13	A.B.14	A.B.15	A.B.16	A.B.17	A.B.18

Seament	Number	Number in Variant Group	t Group	Segment Number in Variant Group	Number	in Varian	t Group
0	Variant	Variant	Variant	آ و	Variant	Variant	Variant
	_	7	2		-	7	-
A.α.1	4	우	0	A.B.1	0	0	မ
Α.α.2	12	2	0	Α.β.2	0	0	S.
Α.α.3	9	-	0	A.B.3	4	0	0
Α.α.4	0	•	0	A.B.4	0	0	-
Α.α.5	0	-	0	A.B.5	0	0	-
Α.α.6	-	0	0	A.B.6	0	0	-
A.a.7	-	0	0	A.B.7	0	0	-
Α.α.8	₩.	0	0	A.β.8	0	0	-
Α.α.9	-	0	0	A.B.9	-	0	0
A.α.10	-	0	0				
A.a.11	<del></del>	0	0				
A.α.12	-	0	0				
A.a.13	-	0	0				
A.a.14	-	0	0				
A.α.15	-	0	0				
A.α.16		0	0				

Fig. 2B: Table 8. Segment B. Unique sequences in fHbb variable segment B and number from each variant group.

	Number in variant of the No.		.CIA CII CII CII CII CII CII CII CII CII	Nun	Number in variant group	dno
Segilleili ID	eguenhec	<u>5</u>		Variant 1	Variant 2	Variant 3
B.α.1	IRQIEVDGQL	ITLES	85	28	12	0
Β.α.2	IRQIEVDGKL	ITLES	86	5	-	0
Β.α.3	IRQIEVDGQT	ITLAS	87	0	8	0
Β.α.4	IRQIEVDRQL	ITLES	88	2	0	0
Β.α.5	IRQIEVDGQL	ITLEN	89	-	0	0
Β.α.6	IHQIEVDGQL	ITLES	06	-	0	0
B.α.7	IRQIEVNGQL	ITLES	91	_	0	0
B.α.8	IRQIRSDGQL	ITLES	92			
B.α.9	IRQIEVDGQL	ITLER	93			
B.α.10	IRQIEVDGRL	ITLES	94			
R R 1	VORTEVECT	TTT.A S	95	U	C	16

GS GS SS GS GS GS GS SS GRATYRGTAF GRATYRGTAF GRATYRGTAF HISFDKLPKD VMATYRGTAF GSATYRGTAF GSATYRGTAL GSATYRGTAF GRATYRGTAL GRATYRGIAF GSAIYRGTAF GRATYRGIAF GSATYRGTAF VMATYRGTAF VMATYRGTAF GRATYRGIAF HISFDKLPEG GRATYRGTAF VMATYRGTAF SSATYRGIAF GRATYRGTAF DRATYRGTAF DSATYRGTAF GSATYRGTAF GRATYRGTAF GSATYRGTAF VMATYRGTAF GRATYRGTAF HISFDKLPKD HISFDKLPKG HISFDKLPES HISFDKLPKG HISFGKLPKD HISFDKLPKG HISFDKLPKG HISFDKLPKD HISFDKLPKG HISFDKLREG VYKQSHSALT ALQTEQVQDS EDSGKMVAKR QFRIGDIAGE HTSFDKLPKG HISFDKLPKG VYKQSHSALT ALQTEQVQDS EHSGKMVAKR QFRIGDIAGE HTSFDKLPEG HISFDKLPEG HISFDKLPEG HISFDKLPEG HISFDKLPKD HISFDKLPKG HISFDKLPEG HIFFDKLPKG HISFDKLPEG HTSFDKLPKG HISFDKLPEG HISFDKLPEG ALQIEQVQDS EHSRKMVAKR QFRIGDIAGE EHSGKMVAKR QFRIGDIAGE EDSGKMVAKR QFRIGDIAGE VYKQSHSALT ALQTEQVQDS EHSGKMVAKR QFRIGDIAGE RFRIGDIAGE Fig. 2C: Table 8, Segment C, i. Unique sequences in fHbp variable segment C. ALQIEQVQDS EHSGKMVAKR RFRIGDIAGE QFRIGDIAGE RFRIGDIAGE RFRIGDIAGE OFRIGDIAGE OFRIGDIAGE QFRIGDIAGE RFKIGDIAGE QFRIGDIVGE RFRIGDIAGE RFRIGDIAGE EHSGKMVAKR QFRIGDIVGE RFRIGDIAGE EHSGKMVAKR QFRIGDIAGE VYKQSHSALI ALQIEQVQDS EDSGKMVAKR QFRIGDIAGE EHSGSMVAKR QFRIGDIAGE QFRIGDIAGE EHSGKMVAKR QFRIGDIAGE RFRIGDIAGE Sequence EHSGKMVAKR EHSEKMVAKR EHSGKMVAKR EHSRKMVAKR EHSGKMVAKR EHSGKMVAKR EHSGKMVAKR EDSGKMVAKR EHSGKMVAKR EHSGKMVAKR EHSGKMVAKR EHSGKMVAKR EHSGKMVAKR VYKQSHSALI ALQTEQVQDS EDSGKMVAKR EDSGKMVAKR **AFQTEQIQDS** VYKOSYSALT ALQTEQIQDS AFQTEQIQDS VYKQSHSALT AFQTEQIQDS VYKQSHSALT ALQTEQVQDS ALQTEQEQDS VYKQSHSALT ALQTEQEQDL ALQTEQVQDS VYKQSHSALT AFQTEQIQDS ALQTEQIQDS ALQTEQVQDS VYKQSHSALT ALQTEQVQDS VYKQSHSALT AFQTEQIQDS VYKQSHSALT ALQTEQVQDS JYKQSHSALT ALQTEQEQDP VYKQSHSALT ALQTEQVQDS VYKQSHSALT ALQTEQEQDP VYKOSHSALT ALQTEQVQDS VYKQSHSALT AFQTEQIQDS VYKQSHSALT ALQTEQVQDS ALOTEQVODS VYKQSHSALT VYKQSHSALT /YKQSHSALT VYKQSHSALT VYKQSHSALT VYKQSHSALT VYKQSHSALT VYKOSHSALT VYKQSYSALT Segment C.a. 15 C.a. 16  $C.\alpha.19$ C.a.20  $C.\alpha.17$ C.a. 18 C.a.12 C.a.13 C.a.14 C.a.21 C.a.22 C.a.23 C.a.24 C.a.25 C.a.26 C.a.28 C.a.10 C.a.11 C.α.9 C.a.6 C.a.8  $C.\alpha.5$ C.a.7 C.a.2 C.a.3 C. a. 4

C.a.29	C.a.29 VYKQSHSALT	ALQTEQVQDS	EDSGKMVAKR	T ALQTEQVQDS EDSGKMVAKR QFRIGDIAGE HISFI	HISFDKLPKG	DSATYRGTAF		124
C.a.30	VYKQSHSALT	ALQTEQVQDS	EDSGKMVAKR	QFRIGDIAGE	HISFDKLPES	DSATYRGTAF	SS	125
C.a.31	VYKQSHSALT	ALQTEQVQDS	EDSGKMVAKR	QFRIGDIAGE	HISFDKLPKG	GRATYRGIAF	GS	126
.32	VYKQSHSALT	ALQTEQVQDS	EDSGKMVAKR	QFRIGDIAGE	HISFDKLHKG	GSATYRGTAF	SS	127
C.a.33	VYKQSHSALT	ALQTEQVQDS	EDSRKMVAKR	QFRIGDIAGE	HISFDKLPES	DRATYRGTAF	SS	128
C.a.34	VYKQSHSALT	ALQTEQEQDP	EHSGKMVAKR	RFKIGDIAGE	HISFDKLPKD	VMVTYRGTAF	GS	129
C. a.35	VYKQSHSALT	ALQTEQVQDS	EHSAKMVAKR	QFRIGDIAGE	HISFDKLPEG	GRATYRGTAF	GS	130
C.a.36	VYKQSHSALT	ALQTEQEQDP	EHSEKMVAKR	RFKIGDIAGE	HISFDKLPKD	VMATYRGTAF	GS	131
C.a.37	VYKQSHSALT	ALQTEQVQDS	EHSEKMVAKR	RFKIGDIAGE	HISFDKLPKD	VMATYRGTAF	GS	132
C.a.38	VYKQSYSALT	ALQTEQVQDS	EDSGKMVAKR	QFRIGDIAGE	HISFDKLPKG	GSATYRGTAF	GS	133
C.a.39	VYKQSYSALT	ALQTEQVQDS	EHSGKMVAKR	RFRIGDIAGE	HISFDKLPGD	SRATYRGTAF	gs	134
40	VYKQSHSALT	ALQTEQVQDS	EHSGKMVAKR	RFRIGDIAGE	HISFDKLPEG	GRATYRGTAF	SS	135
14	VYKQSHSALT	ALQTEQVQDS	EDSGKMVAKR	QFRIGDIAGE	HISFDKLPED	VRATYRGTAF	GS	136
C.α.42	VYKQSHSALT		EHSGKMVAKR	QFRIGDIAGE	HISFDKLPEG	VRATYRGTAF	GS	137
C.a.43	VYKQSHSALT	AFQTEQEQDS	EHSGKMVAKR	QFRIGDIAGE	HISFDKLPDV	VRATYRGTAF	GS	138
C.a. 44	VYKQSHSALT	ALQTEQVQDS	EHSGKMVAKR	QFRIGDIAGE	HISFDKLPES	GRATYRGTAF	GS	139
C.a.45	VYKQSHSALT	ALQTEQEQDL	EHSGKMVAKR	RFKIGDIAGE	HISFDKLPKD	VMATYRGTAF	GS	140
C. a. 46	VYKQSHSALT	ALQTEQVQDS	EHSEKMVAKR	QFRIGDIAGE	HISFDKLPES	GSATYRGTAF	GS	141
C.a.47	VYKQSHSALT	AFQTEQVQDS	EHSGKMVAKR	QFRIGDIAGE	HISFDKLPEG	GRATYRGTAF	GS	142
C.a.48	VYKQSHSALT	ALQTEQVQDS	EHSGKMVAKR	QFRIGDIAGE	HISFDKLPKG	VRATYRGTAF	GS	143
C.a.49	VYKQSHSALT	ALQTEQEQDL	EHSRKMVAKR	RFKIGDIAGE	HISFDKLPKD	VMATYRGTAF	GS	144
C.a.50	VYKQSHSALT	ALQTEQEQDL	EHSRKMVAKR	RFKIGDIAGE	HISFDKLPKD	VMATYRGTAF	GS	145
C.a.51	VYKQSHSALT	ALQTEQEQDP	EHSGKMVAKR	RFKIGDIAGE	HISFDKLPKD	IMATYRGIAF	GS	146
C.a.52	VYKQSHSALT	ALQTEQVQDS	EHSGRMVAKR	QFRIGDIAGE	HISFDKLPEG	GRATYRGIAF	GS	147
C.a.53	VYKQSHSALT	ALQTEQVQDS	EHFGKMVAKR	QFRIGDIAGE	HISFDKLPEG	GRATYRGIAF	GS	148
C. 0.54	VYKQSHSALT	ALQTEQVQDS	EHSGKMVAKR	RFRIGDIAGE	HISFDKLPKD	GMATYRGIAF	GS	149
C. 0.55	IYKQSHSALT		EHSGKMVAKR	QFRIGDIAGE	HISFDKLPEG	GRATYRGIAF	GS	150
C o 56	VYKOSHSALT	•	EDSGKMVAKR	RFKIGDIAGE	HISFDKLPKD	VMATYRGTAF	GS	151
	IYKODHSAVV		DKIDSLINQR	SFLVSGLGGE	HTAFNQLP-G	GKAEYHGKAF	SS	152
C.B.2	IYKQDHSAVV		DKIDSLINQR	SFLVSGLGGE	HTAFNQLP-S	GKAEYHGKAF	SS	153
	ł						0	127

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Fig. 2C (C	Fig. 2C (Con't): Table 8, Segment C, i. Unique sequences in tHbp variable segment C.	egment C, i. Unid	due sedneuces in	tHbp variable se	gment C.			
C.B.4	IYKQDHSAVV	ALQIEKINNP	DKIDSLINOR	SFLVSGLGGE	SFLVSGLGGE HTAFNQLP-D GKAEYHGKAF	GKAEYHGKAF	28	155
C.B.5	IYKONHSAVV		DKIDSLINQR	SFLVSGLGGE	HTAFNQLP-G GKAEYHGKAF	GKAEYHGKAF	SS	156
C.B.6	IYKQDHSAVV		DKIDSLINQR	SFLVSGLGGE	HTAFNQLP-S	GKAEYHGKAF	SF	157
C.8.7	IYKQDHSAVV	ALQIEKINNP	DKIDSLINRR	SFLVSGLGGE	HTAFNQLP-D	GKAEYHGKAF	SS	158
C. B.8			DKIDSLINQR	SFLVSGLGGE	HTAFNQLP-V	GKAEYHGKAF	SS	159
0.8.9			DKIDSLINQR	SFLVSGLGGE	HTAFNQLP-G	GKAEYHGKAF	SS	160
C.B.10		ALQIEKINNP	DKIDSLINQR	SFLVSGLGGE	HTAFNQLP-G	GKAEYHGKAF	SS	161
C.B.11	IYKQNHSAVV	ALQIEKINNP	DKIDSLINQR	SFLVSGLGGE	HTAFNQLP-G	DKAEYHGKAF	28	162
C.B.12	IYKODHSAVV	ALQIEKINNP	DKIDSLINQR	SFLVSGLGGE	HTAFNQLP-V	DKAEYHGKAF	SS	163
C.B.13	IYKOGHSAVV	ALQIEKINNP	DKIDSLINQR	SFLVSGLGGE	HTAFNQLP-S	GKAEYHGKAF	SS	164
C.B.14	IYKODHSAVV	ALQIEKINNP	DKIDSLINQR	SFLVSGLGGE	HTAFNQLP-S GKAEYHGKAL	GKAEYHGKAL	SS	165
C.B.15	IYKODHSAVV	ALQIEKINNP	DKIDSLINQR	SFLVSGLGGE	HTAFNQLP-G	DKAEYHGKAF	SS	166
C.B.16	IYKODHSAVV	ALOTEKVNNP		SFLVSGLGGE	HTAFNQLP-V GKSEYHGKAF	GKSEYHGKAF	SS	167
C.B.17	IYKONHSAVV	ALQIEKINNE	DKIDSLINQR	SFLVSGLGGE	HTAFNQLP-S	GKAEYHGKAF	SS	168
C.B.18	IYKONHSAVV	ALOIEKINNE	DKIDSLINOR	SFLVSSLGGE	HTAFNQLP-G	GKAEYHGKAF	SS	169
C.B.19	VYKODHSAVV	ALO	DKIDSLINOR	SFLVSGLGGE	HTAFNQLP-G	GKAEYHGKAF	SS	170
C.B.20	IYKQDHSAVV		DKTDSLINQR	SFLVSGLGGE	HTAFNQLP-G	GKAEYHGKAF	SS	171
C.B.21	IYKQDHSAVV		DKIDSLINQR	SFLVSGLGGE	HTAFNQLS-G	GKAEYHGKAF	SS	172
C.B.22	IYKQDHSAVV		DKIDSLINQR	SFLVSGLGGK	HTAFNQLP-G	GKAEYHGKAF	SS	173

Fig. 2D: Table 8, Seg	able 8, Seg	gment C, i	i. Unique	sequences in	fHbp vari	able segm	ent C and n	ment C, ii. Unique sequences in fHbp variable segment C and number from each variant group.
Segment Number	Number	in Variant Group	Group	Segment	Number in Variant Group	in Varian	t Group	
<u>.</u>	Variant	Variant	Variant	_ 	Variant	Variant	Variant	
	<del>-</del>	2	က		_	2	3	
C.a.1	မ	0	0	C.B.1	0	2	80	
C.a.2	4	0	0	C.B.2	0	9	α	
C.a.3	က	0	0	C.B.3	0	α	0	
C.α.4	ო	0	0	C.B.4	0	က	0	
C.a.5	7	0	0	C.B.5	0	0	2	
C.α.6	က	0	0	C.B.6	0	-	0	
C.a.7	2	0	0	C.B.7	0	-	0	
C.α.8	_	0	0	C.β.8	0	0	-	
C.α.9	_	0	0	С.в.9	0	0	-	
C.a. 10	-	0	0	C.B.10	0	0	-	
C.a.11	-	0	0	C.B.11	0	0	-	
C.α.12	•	0	0	-				
C.a.13	-	0	0					
C.a.14	_	0	0					
C.a.15	-	0	0					
C.a.16	-	0	0					
C.a.17	-	0	0					
C.a. 18	-	0	0					
C.a.19	-	0	0					
C.a.20	-	0	0					
C.a.21	-	0	0					
C.a.22	-	0	0					

Fig. 2D (Con't):	Fig. 2D (Con't): Table 8, Segment D. Unique sequences in tHbp variable segment D and number from each variant group	Unique sequences in	thbp variable segr	nent D and numbe	r from each variant	group.
	(	!		IN.	Number in variant group	dno
Segment ID	nbes	sednence	SEC ID NO.	Variant 1	Variant 2	Variant 3
D.α.1	AGGKLIYIID	FAAKQGHGK	174	21	6	6
D.a.2	AGGKLIYTID	FAAKQGHGK	175	S	0	0
D. a. 3	ASGKLTYTID	FAAKQGHGK	176	2	0	0
D.α.4	AGGKLTYTID	FAAKQGYGK	177	α	0	0
D.a.5	AGGKLIYIID	FAAKQGNGK	178	7	0	0
D.a.6	AGGKLIYTID	FAVKQGHGK	179	α	0	0
D.a.7	AGGKLTYTID	FATKQGHGK	180	0	· ·	0
D.a.8	AGGKLTYTID	FASKQGHGK	181	0	0	•
D.α.9	AGGKLIYTID	FAAKQGYGK	182	-	0	0
D.a.10	ARGKLTYTID	FAAKQGHGK	183			
D.α.11	ASGELTYTID	FAAKQGHGK	184			
D.a.12	IGGKLTYTID	FAAKQGHGK	185			
D.a.13	AGGKLTYTID	FVAKQGHGK	186			
D.α.14	AGGKLTYTID	FAAKQGHGR	187			
D.a.15	AGGRLTYTID	FAAKQGCGK	188			
D.α.16	AGEKLTYTID	FAAKQGHGK	189			
D.a.17	TGGKLIYTID	FAAKQGHGK	190			
D.a.18	AGGKLTYIID	FAAKQGYGK	191			
D.B.1	PNGRLHYSID	FIKKQGYGR	192	0	2	7
0.8.2	PNGRLHYTID	FINKQGYGR	193	0	0	က
D.B.3	PNGRLHYSID	FTKKQGYGG	194	0	0	•

Fig. 2E: Table 8.		nt E, i. Unique se	Segment E, i. Unique sequences in fHbp variable segment E.	variable segmen	t E.			
Segment				Sequence				SEQ II
Ε.α.1	SPELNVDLAA	AYIKPDEKHH	AVISGSVLYN	QAEKGSYSLG	IFGGKAQEVA	GSAEVKTVNG	IRHIGLAA	195
E.α.2	SPELNVDLAA	SDIKPDKKRH	AVISGSVLYN	QAEKGSYSLG	IFGGQAQEVA	GSAEVETANG	IRHIGLAA	196
E.a.3	SPELNVELAT	AYIKPDEKHH	AVISGSVLYN	QDEKGSYSLG	IFGGQAQEVA	GSAEVETANG	IHHIGLAA	197
Ε.α.4	SPELNVDLAA	ADIKPDEKHH	AVISGSVLYN	QAEKGSYSLG	IFGGQAQEVA	GSAEVETANG	IRHIGLAA	198
E.α.5	SPELNVDLAA	ADIKPDEKRH	AVISGSVLYN	QAEKGSYSLG	IFGGKAQEVA	GSAEVKTVNG	IRHIGLAA	199
E.α.6	SPELNVELAT	AYIKPDEKRH	AVISGSVLYN	QDEKGSYSLG	IFGGQAQEVA	GSAEVETANG	IHHIGLAA	200
Εα.7	SPELNVDLVS	ADIKPDEKRH	AVISGSVLYN	QDEKGSYSLG	IFGGKAQEVA	GSAEVKTVNG	IRHIGLAA	201
Щ. 8.8	SPELNVDLAA	ADIKPDGKRH	AVISGSVLYN	QAEKGSYSLG	IFGGKAQEVA	GSAEVKTVNG	IRHIGLAA	202
Б. В.		ADIKPDGKRH	AVISGSVLYN	QAEKSSYSLG	IFGGKAQEVA	GSAEVKTVNG	IRHIGLAA	203
E.α.10	SPELNVDLAA	ADIKPDEKHH	AVISGSVLYN	QAEKGSYSLG	IFGGKAQEVA	GSAEVKTVNG	IRHIGLAA	204
E.a.11		ADIKPDEKHH	AVISGSVLYN	QDEKGSYSLG	IFGGKAQEVA	GSAEVKTVNG	IRHIGLAA	202
E.a. 12	SPELNVDLAA	ADIKPDEKHH	AVISGSVLYN	QDEKGSYSLG	IFGGEAQEVA	GSAEVKTANG	IHHIGLAA	206
E.a. 13	SPELNVDLAV	AYIKPDEKHH	AVISGSVLYN	QDEKGSYSLG	IFGEKAQEVA	GSAEVKTANG	IHHIGLAA	207
E.α.14	SPELNVDLAV	AYIKPDEKHH	AVISGSVLYN	QDEKGSYSLG	IFGEKAQEVA	GSAEVETANG	IHHIGLAA	208
E. α. 15	SPELNVDLAA	ADIKPDEKHH	AVISGSVLYN	QDEKGSYSLG	IFGGQAQEVA	GSAEVETANG	IHHIGLAA	209
E.α.16	SPELNVDLAA	AYIKPDKKRH	AVISGSVLYN	QDEKGSYSLG	IFGGQAQEVA	GSAEVETANG	IHHIGLAA	210
E.α.17	SPELNVDLAA	AYIKPDEKHH	AVISGSVLYN	QDEKGSYSLG	IFGGQAQEVA	GSAEVKTANG	IRHIGLAA	211
E 0. 18	SPELNVELAA	AYIKPDEKHH	AVISGSVLYN	QDEKGSYSLG	IFGGQAQEVA	GSAEVETANG	IQHIGLAA	212
19		ADIKPDEKHH	AVISGSVLYN	QDEKGSYSLG	IFGGQAQEVA	GSAEVETANG	IHHIGLAA	213
E. a. 20	SPELNVELAT	AYIKPDEKHH	AVISGSVLYN	QDEKGSYSLG	IFGGKAQEVA	GSAEVETANG	IHHIGLAA	214
E.α.21		AYIKPDEKRH	AVISGSVLYN	QDEKGSYSLG	IFGGQAQEVA	GSAEVKTVNG	IRHIGLAA	215
E.α.22	SPELNVELAT	AYIKPDEKRH	AVISGSVLYN	QDEKGSYSLG	IFGGQAQEVA	GSAEVETANG	IRHIGLAA	216
E.α.23	SPELNVELAT	AYIKPDEKRH	AVISGSVLYN	QDEKGSYSLG	IFGGQAQEVA	GSAEVETANG	IQHIGLAA	217
E.α.24	SPELNVELAT	AYIKPDEKRH	AVISGSVLYN	QDEKGSYSLG	IFGGQAQEVA	GSAEVETVNG	IHHIGLAA	218
E a 25	SPELNVNLAA	ADIKPDEKRH	AVISGSVLYN	QDEKGSYSLG	IFGEKFQEVA	GSAEVKTANG	IHHIGLAA	219
E.α.26	SPELNVDLAA	AYIKPDEKHY	AVISGSVLYN	QDEKGSYSLG	IFGGKAQEVA	GSAEVKTVNG	IRHIGLAA	220
E.α.27	SPELNVDLAA	AYIKPDEKRH	AVISGSVLYN	QDEKGSYSLG	IFGGKAQEVA	GSAEVKTANG	IHHIGLAA	221
E.α.28	SPELNVDLAA	SDIKPDKKRH	AVISGSVLYN	QAEKGSYSLG	IFGGQAQEVA	WQRRVETANG	IRHIGLAA	222
E.α.29	SPELNVDLAA	ADIKPDEKHH	AVISGSVLYN	QDEKGSYSLG	IFGGKAQEVA	GSAEVETANG	IHHIGLAA	223

Fig. 2F (1	On't) . Table 8	Ria 2F (Con't) · Table & Segment F. i. Unique sequences in fHbp variable segment E	Injoue sequences	in fHbp variable	segment E.			
Segment		,		Sequence				SEQ ID
Ε.α.30	SPELNVDLAA	SDIKPDKKRH	AVISGSVLYN	QAEKGSYSLG	IFGRQAQEVA	GSAEVETANG	IRHIGLAA	224
E.α.31	SPELNVDLAA	SDIKPDKKRH	AVISGSVLYN	QAEKGSYSLG	IFGGKAQEVA	GSAEVETANG	IRHIGLAA	225
E.a.32	SPELNVDLAA	ADIKPDKKRH	AVISGSVLYN	QAEKGSYSLG	IFGGQAQEVA	GSAEVETVNG	IRHIGLAA	226
E.α.33	SPELNVDLAA	ADIKPDEKRH	AVISGSVLYN	QDEKGSYSLG	IFGGQAQEVA	GSAEVETANG	IQHIGLAA	227
E.α.34	SPELNVELAA	AYIKPDEKRH	AVISGSVLYN	QDEKGSYSLG	IFGGQAQEVA	GSAEVETANG	IQHIGLAA	228
E.α.35	SPELNVELAT	AYIKPDEKHH	AVISGEVLYN	QDEKGSYSLG	IFGGQAQEVA	GSAEVETANG	IHHIGLAA	229
E.α.36	SPELNVDLAA	AYIKPDEKRH	AVISGSVLYN	QDEKGSYSLG	IFGGKAQEVA	GSAEVKTVNG	IRHIGLAA	230
E.α.37	SPELNVDLAA	SDIKPDKKRH	AVISGSVLYN	HAEKGSYSLG	IFGGQAQEVA	GSAEVETANG	IRHIGLAA	231
E.a.38	SPELNVDLAA	AYIKPDEKRH	AVISGSVLYN	QDEKGSYSLG	IFGGQAQEVA	GSAEVETANG	IQHIGLAA	232
F 0.39	SPEL,NVDL,VA		AVISGSVLYN	QAEKGSYSLG	IFGGQAQEVA	GSAEVETANG	IRHIGLAA	233
E.α.40	SPELNVELAT		AVISGSVLYN	QDEKGSYSLG	IFSGQAQEVA	GSAEVETANG	IHHIGLAA	234
E.α.41	SPELNVELAT		AVISGSVLYN	QDEKGSYSLG	IFGGQAQEVA	GSAEVETANG	IHHIGLAA	235
E.α.42	SPELNVDLAV		AVISGSVLYN	QDEKGSYSLG	IFGGQAQEVA	GSAEVETANG	IHHIGLAA	236
E.α.43	SPELNVELAT		AVISGSVLYN	QDEKGSYSLG	IFGGQAQEIA	GSAEVETANG	IHHIGLAA	237
E.α.44	SPELNVDLAA		AVISGSVLYN	QDEKGSYSLG	IFGEKAQEVA	GSAEVKTANG	IRHIGLAA	238
E.a.45	SPELNVELAT	AYIKPDEKRH	AVISGSVLYN	QNEKGSYSLG	IFGGQAQEVA	GSAEVETANG	IHHIGLAA	239
E.α.46	SPELNVELAA	AYIKPDEKRH	AVISGSVLYN	QDEKGSYSLG	IFGGQAQEVA	GSAEVETANG	IHHIGLAA	240
E.a.47	SPELNVDLAV	AYIKPDEKHH	AVISGSVLYN	QDEKGSYSLG	IFGEKAQEVA	GSAEVKTVNG	IRHIGLAA	241
F 0.48	SPELNVDLAV		AVISGSVLYN	QDEKGSYSLG	IFGGKAQEVA	GSAEVETANG	IHHIGLAA	242
F 2 49	SPET.NVDI,AA		AVISGSVLYN	ODEKGSYSLG	IFGGQAQEVA	GSAEVETANG	IHHIGLAA	243
F 0.50	SPELNVDLAA		AVISGSVLYN	QAEKGSYSLG	IFGGQAQEVA	GSAEVKTVNG	IRHIGLAA	244
E.a.51	SPELNVNLAA		AVISGSVLYN	QAEKGSYSLG	IFGEKAQEVA	GSAEVKTANG	IHHIGLAA	245
E.α.52	SPELNVDLAA	AYIKPDEKHH	AVISGSVLYN	QDEKGSYSLG	IFGGKAQEVA	GSAEVETVNG	IRHIGLAA	246
E. c. 53	SPELNVDLAA	ADIKPDEKRH	AVISGSVLYN	QDEKGSYSLG	IFGGKAQEVA	GSAEVETANG	IQHIGLAA	247
E.α.54	SPELNVDLAA	ADIKPDEKHH	AVISGSVLYN	QDEKGSYSLG	IFGEKAQEVA	GSAEVKTANG	IHHIGLAA	248
E. 0.55	SPELNVDLAA		AVISGSVLYN	QDEKGSYSLG	IFGGKAQEVA	GSAEVKTVNG	IRHIGLAA	249
E.α.56	SPELNVDLAA	ADIKPDEKRH	AVISGSVLYN	QDEKGSYSLG	IFGGQAQEVA	GSAEVKTVNG	IRHIGLAA	250
$E.\alpha.57$	SPELNVDLAA	AYIKPDEKHH	AVISGSVLYN	QDEKGSYSLG	IFGGQAQEVA	GSAEVETANG	IQHIGLAA	251
E.a.58	SPELNVDLAA	A AYIKPDKKRH	AVISGSVLYN	QDEKGSYSLG	IFGGKAQEVA	GSAEVETANG	IHHIGLAA	252
E.α.59	SPELNVDLAA	AYIKPDEKHH	AVISGSVLYN	QAEKGSYSLG	IFGGQAQEVA	GSAEVETANG	IRHIGLAA	253
E.α.60	SPELNVELAT	I AYIKPDEKRH	AVISDSVLYN	QDEKGSYSLG	IFGGQAQEVA	GSAEVETANG	IHHIGLAA	254

Fig. 2E (Con't): Table 8, Segment E, i. Unique sequences in fHbp variable segment E.

FIG. 4L.	Fig. 2E (Con't): Table 8, Segment E, 1. Unique sequences in I Hop variable segment E.	, Segment E, 1.	Juidne sednences	s in thop variable	e segment E.			
Segment ID				Sequence				SEQ ID NO:
E.α.61	SPELNVDLAA	AYIKPDEKHH	AVISGSVLYN	QDEKGSYSLG	IFGGKAEEVA	GSAEVKTVNG	IRHIGLAA	255
E.α.62	SPELNVDLAA ANIEQ	ANIEQDEKHH	AVISGSVLYN	QAEKGSYSLG	IFGEKFQEVA	GSAEVKTVNG	IRHIGLAA	256
E. a. 63	SPELNVDLAA	AYIKPDEKHH	AVISGSVLYN	QAEKGSYSLG	IFGGKAQEVA	GSAEVKTVNG	ILHIGLAA	257
E.α.64	SPELNVELAT	AYIKPDEKHH	AVISGSVLYN	QDEKGSYSLG	IFGGQAQEVA	GSAEVETANG	IRHIGLAA	258
E. 065	SPELNVDLAA	ADIKPDEKHH	AVISGSVLYN	QDEKGSYSLG	IFGGQAQEVA	GSAEVKTVNG	IRHIGLAA	259
E. a. 66	SPELNVDLAA	SDIKPDKKRH	AVISGSVLYN	QAEKGSYSLG	IFGGQAQEVA	GSAEVKTVNG	IRHIGLAA	260
$E.\alpha.67$	SPELNVDLAA	ADIKPDEKRH	AVISGSVLYN	QDEKGSYSLG	IFGGQAQEVA	GSAEVETANG	IHHIGLAA	261
E.8.1	TPEQNVELAS	AELKADEKSH	AVILGDTRYG	GEEKGIYHLA	LFGDRAQEIA	GSATVKIREK	VHEIGIAG	262
E.8.2	TPEQNVELAA		AVILGDTRYG	SEEKGIYHLA	LFGDRAQEIA	GSATVKIGEK	VHEIGIAG	263
E.8.3	TPEQNVELAS		AVILGDTRYG	SEEKGIYHLA	LFGDRAQEIA	GSATVKIREK	VHEIGIAG	264
E.8.4	TPEQNVELAS	AELKADEKSH	AVILGDTRYG	SEEKGTYHLA	LFGDRAQEIA	GSATVKIGEK	VHEIGIAG	265
E.8.5	TPELNVDLAS	AELKADEKSH	AVILGDTRYG	SEEKGTYHLA	LFGDRAQEIA	GSATVKIGEK	VHEIGIAG	566
E.8.6	TLEQNVELAA	AELKADEKSH	AVILGDTRYG	SEEKGTYHLA	LFGDRAQEIA	GSATVKIGEK	VHEIGIAG	267
E.B.7	TPEQNVELAA	AELKADEKSH	AVILGDTRYG	GEEKGTYHLA	LFGDRAQEIA	GSATVKIREK	VHEIGIAG	268
E.8.8	TPEQNVELAA	AELKADEKSH	AVILGDTRYD	SEEKGIYHLA	LFGDRAQEIA	GSATVKIGEK	VHEISIAG	569
E.B.9	TPEQNVELAA		AVILGDTRYG	SEEKGIYHLA	LFGDRAQEIA	GSATVKIGEK	VHEISIAG	270
E.B.10	SPELNVELAT		AVILGDIRYG	SEEKGIYHLA	LFGDRAQEIA	GSATVKIREK	VHEIGIAG	271
E.B.11	TPEQNVELAS	AELKADEKSH	AVILGDTRYG	GEEKGIYHLA	LFGDRAQEIA	GSATVKIREK	VHEIDIAG	272
E.B.12	TPEQNVELAA		AVILGDTHYG	SEEKGIYHLA	LFGDRAQEIA	GSATVKIGEK	VHEISIAG	273
E.B.13	TPEQNVELAA		AVILGDTRYG	SEEKGIYHLA	LFGDRAQEIA	GSATVKIREK	VHEIGIAG	274
E.B.14	TPEQNVELAS	AELKADEKSH	AVILGDTRYG	GEEKGIYHLA	LFGDRAQEIA	GSATVKIGEK	VHEIGIAG	275
E.B.15	TPEQNVELAS	AELKADEKSH	AVILGDTRYG	SEEKDIYHLA	LFGDRAQEIA	GSATVKIREK	VHEIGIAG	276
E.B.16	TPELNVDLAS		AVILGDTRYG	GEEKGIYHLA	LFGDRAQEIA	GSATVKIREK	VHEIGIAG	277

Fig. 3

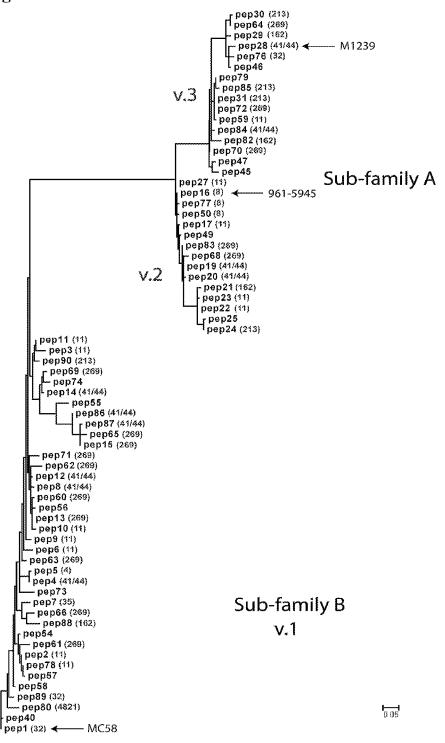
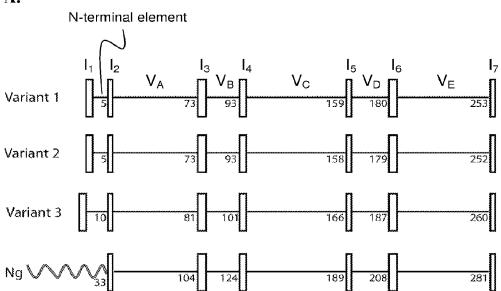


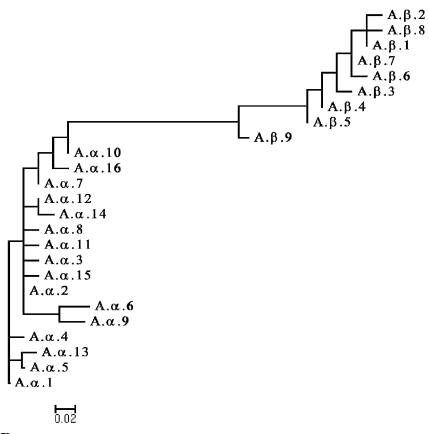
Fig. 4 A.



В.

Invariant segment	Amino acid residues
$I_1$	CSSG (SEQ ID NO: 2)
$I_2$	GG
$I_3$	SRFDF (SEQ ID NO: 3)
$I_4$	GEFQ (SEQ ID NO: 4)
$I_5$	DD
$I_6$	IEHLK (SEQ ID NO: 5) or IEHLE
	(SEQ ID NO: 6)
l <sub>7</sub>	KQ

Fig. 5 A.



## B.

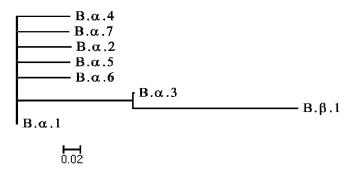
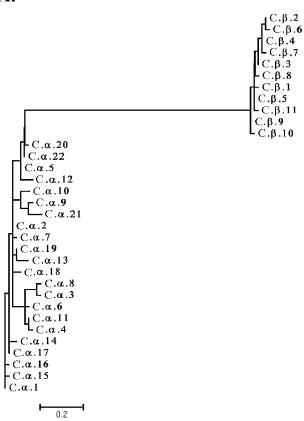
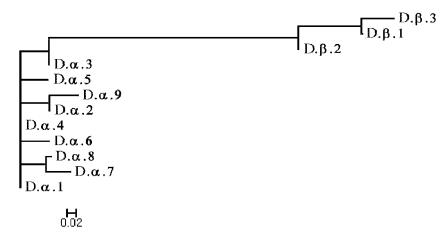


Fig. 6 A.



## B.



**Fig.** 7

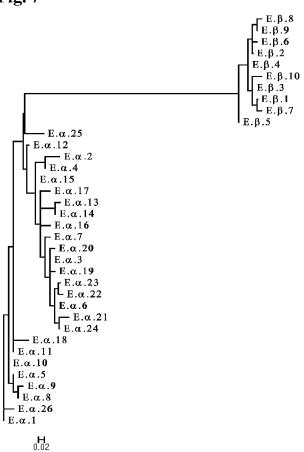
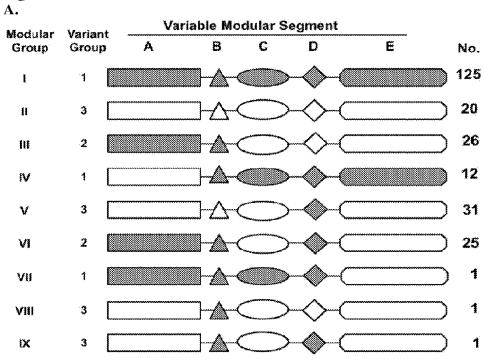
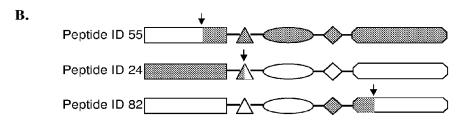


Fig. 8

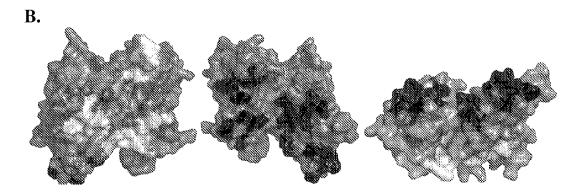




<b>C.</b>		
Variable segment	Conserved junction point within the variable segment	Amino acid residue(s)
Α	J <sub>1</sub>	AQGAE (starting at 50)
		(SEQ ID NO: 278)
В	$J_2$	IEV (starting at 82)
E	$J_3$	A (196)

Fig. 9





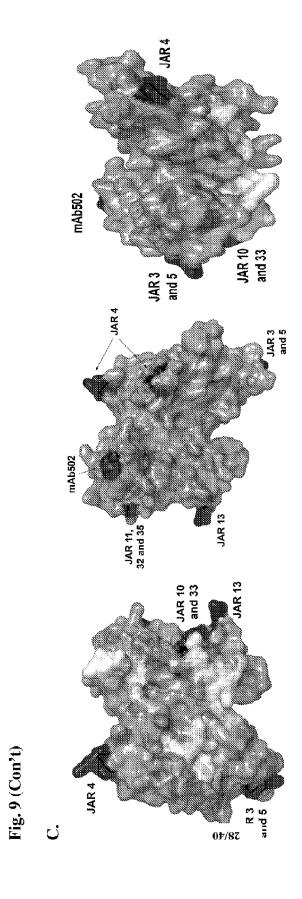


Fig. 10A: Table 9, Characteristics of unique factor H binding protein variants.

	C-terminal <sup>6</sup>																												
		H	Ea8	1%1	$E\alpha I$	$E\alpha 2$	Ea2	Fa17	Ea12	Ea23	Ea16	Ea3	Ea18	9¤3	$E\alpha 6$	Ea3	$E\alpha4$	EB2	EB2	EB13	Eß1	EB1	ega	EB1	EB1	EB1	EB1	Eβ14	Eß2
	nt Allele <sup>5</sup>	q	ς¤Ω	1)¤3	$D\alpha 1$	$\Sigma^{0}\Omega$	$\varepsilon$ $\alpha$ C	D¤4	$D\alpha 4$	$D\alpha 1$	9¤Q	$D\alpha 1$	$D\alpha 1$	$D\alpha 1$	Da1	Da1	Da1	$D\alpha 1$	$D\alpha I$	Da1	$D\alpha 1$	$D\alpha 1$	$1$ g $\alpha$	180	$1 g \alpha$	1βα	Iβα	DB1	Dal
	iable Segme	၁	Ca5	Ca1	Ca6	$C\alpha 1$	$C\alpha 1$	Ca14	Ca10	Ca16	Ca6	Ca2	Ca6	Ca2	Ca2	Ca3	$C\alpha 4$	Cβ4	$C\beta 1$	Cβ4	$C\beta2$	CB6	$C\beta 1$	CB2	$C\beta 2$	CB3	CB3	CB3	Сβ4
	Modular Variable Segment Allele <sup>s</sup>	В	Bal	Ba1	Bal	Bα1	$\mathbf{B}a1$	Ba7	Ba1	Ba1	Bal	Ba2	Bal	Bα1	Ba2	Ba1	$\mathbf{B}a1$	$\mathbf{B}\alpha 1$	$\mathbf{B}a1$	Βα10	Bα1	$\mathbf{B}$ $\alpha$ 1	$\mathbf{B}a1$	Ba1	$\mathbf{B}a1$	Ba3	Ba3	Ba3	Bα1
ants.		¥	Aa2	Aa3	Aa3	Aa1	Aa13	Aa2	Aa12	Aa2	Aa2	Aa3	Aa3	Aa1	Aa2	Aa1	Αβ3	Aa1	Aa1	Aa36	Aal	Aa1	Aa1	Aa1	Aa2	Aa1	Aa4	Aa27	Aa2
14: Lable 3, Characteristics of unique factor if britaing protein variants	N-terminal Variable	- and merical	G	Ð	9	9	9	Ð	Ð	Ð	GGGSGG	Ð	G	9	G	G	GGGSGG	99	GG	G	G	99	G	99	GG	99	G	9	GG
IACIOI II INII	fHbp Modular Groun <sup>4</sup>	d mo	I	l	I	I	I	1	I	Ι	I	I	I	I	I	I	IV	IA	IΛ	VI	VI	IΛ	Ш	Ш	Ш	Ш	Ш	Ш	VI
es or unique	Wyeth Sub-	ranmy	В	8	В	В	В	<u>~</u>	В	В	В	В	В	В	В	В	В	A	A	A	A	A	A	A	A	A	A	A	A
Characteristic	Novartis Variant Groun <sup>2</sup>	dinorp	1	1	1	1	1	_	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2
A: Lable 2,	fHbp Protein m'	3	1	2	з	4	5	9	7	∞	6	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27

EFI Ea56  $E\alpha 13$ Ea13 Ea13 Ea14 Ea10 Ea24 Ea22 Ea2 Εα9 Ea21 Ea11  $E\alpha 1$  $E\alpha 1$ Dal Dal Dal Dal Dα1 Dα1 Dβ1 Dal Da3  $D\alpha 1$  $\mathrm{D}\alpha 1$ Da7 Da2 Da1  ${\rm D}\alpha 1$  $D\alpha 2$  $D\alpha 8$ Dal Da2 Dal Da4 Da1 Dal Ca15  $C\alpha 19$ Ca18 Ca45 Ca33  $C\alpha 50$ Ca50 Ca5 CB1 CB1 CB3  $C\alpha 17$ Ca2 CB5 Cal  $C\alpha 8$  $C\alpha 7$ Ca2 Ca.1 C<sub>β1</sub> Ba4  $B\alpha 2$ Βα5 Ba2 Bal Ba7 Bal Ba1 Ba1 Bal Bal Bal Βα1 Bal Bal Bal Bal Bal Bal  $B\alpha 1$ Bβ1 Bal Bal Aa38 Aa13  $A\alpha 40$  

 Αα44

 Αα2

 Αα2

 Αβ1

 Αβ1

 Αβ4

 Αα1

 Λα2

 Λα2

 Λβ9

 Λβ9

 Λα10 Λα9 Aa7 Aa3 Aa2  $A\alpha 2$ Λα3 AB2 Λα2 Λα2 Λβ1 Αβ3  $A\alpha 11$  $A\alpha 1$ Fig. 10B: Table 9, Characteristics of unique factor H binding protein variants. GGGSGG 998999 GGGSGG GGGSGG GGGSGS GGGSGG GGGSGS SGSGG 99899 99 0 0 0 Ç G 5 5 ರ G C G G G G G Ç IN IN > M  $\overline{\mathbf{N}}$  $\overline{\mathbf{N}}$  $\equiv$  $\equiv$ > VI  $\forall$ A B B B В ВВ B В  $\mathbf{m}$ m m  $|\mathbf{m}|$  $\forall$  $\forall$ ¥ ¥ V, Þ Ą  $\forall$ A  $\mathbf{R}$  $\mathfrak{A}$  $\mathbf{m}$  $\checkmark$ В 

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Ea19 Eβ2 Fα15 Ea13 EB10 Ea4  $E\alpha 25$ Ea26 Еа18 Еβ9 Еβ1 Εβ3Εβ2Εα1Εβ4Εα7  $E\beta7$ <u>БВ8</u> БВ3 Ea3 | Da1 | Da1 | Da1 | Da1 | DB2 | DB2 | DB1 | DB1 | Da1 Dαθ DB1 Da2 Dal Da4 Da4 Da9 Da1 Da1 Dal Dal Dal  $D\alpha 1$ Dal Dal Dα1  $D\alpha 1$ Dal Dal Dal Ca12 Ca13 CB1 Ca7 Ca20 CB20 CB20 Ca21 Ca22 Ca6 Ca3Ca3 Cβ2 CB2 Ca4 $C\alpha 11$ CB3 CBI CBI C<sub>β</sub>1 Cβ1 CBI  $B\alpha4$  $B\alpha 1$  $B\alpha 1$ Bal  $\frac{B\alpha l}{B\beta l}$ BB1 Ba1 Bal Ββ1 Βα1 Βα1 ВВ1 BB1  $B\alpha 1$ Ββ1 Ββ1  $B\alpha 1$ Ba1 Αβ16 Λα27 Aa14 Λβ3 Λα15 Αα16 Αβ7 Aa2  $A\alpha 2$ Aa3 Λα7 Αβ7 Αα8 Αβ2 Αα6  $\Lambda\beta8$ Λα3 Αβ2  $A\beta2$ Αβ8 Aβ1 Aα1 AβI Aβ1 Fig. 10C: Table 9, Characteristics of unique factor H binding protein variants. 666866GGGSGG GGGSGG GGGSGG <u>666866</u> GGGSGG GGGSGG 998999 GGGSGS GGGSGS GGGSGS GGGSGG GGGS GGGS GGGS GG G Ü G Ü Ü Ü Ü G 0 0 5  $\prod$ M M  $\geq$  $\geq$  $\equiv$  $\equiv$ l⊟ I۸ > Λ > > N B <V m BB  $\forall$ V K Y V ₹  $\forall$ 102 69 71 71 80 78 80 82 888 888 888 888 888 890 94 8 8 6 8 8 72 74 76 LL 101

Eβ2
Eβ3
Eβ9
Eβ9
Eβ9
Fα34 Ea29 EB13 Ea44 Ea3 Ea35  $E\alpha 3$ Εα6 Ea58 Ea38 Ea34 Ea59 Ea23 Ea34 Ea11  $E\alpha 3$ Ea1 Ea8 Еβ1 Eal Eal  $E\beta1$ Dal6 Dal Da12 Da6 Da1 Da2 Da2 Daß Da2 Da5  $\mathrm{D}\alpha 4$ Dal Dα1 Dal  $D_{\alpha}$ Dα1  $\mathrm{D}\alpha 1$  $D\alpha 1$ Dal Dal  $\overline{\mathrm{D}\alpha 1}$  $D\alpha 1$  $D\alpha 1$ Ca2 Ca2 Ca2 CB12 CB2 Ca53 C622 C623 C623 СВ2 СВ2 СВ2 СВ2 CB17  $C_{\alpha 9}$ Ca3  $C\alpha51$  $C\alpha 1$ Сβ4 Ca1 Ba3 Ba1 Ba2 Ba1 Ba1 Ba1 Bal  $B\alpha 1$  $\mathbf{Bal}$  $B\alpha 1$  

 Aα4

 Aα1

 Aα41

 Aα2

 Aα2

 Aα2

 Aa3 Aa3 
 Aa1

 Aa1

 Aa1

 Aa2

 Aa3

 Aa3

 Aa3

 Aa3

 Aa2

 Aa3

 Aa2

 Aa2
 Aα3 Aα1 Λα37 Aα3 Aα1 Aα1 AαΙ Aa11  $A\alpha 1$ Fig. 10D: Table 9, Characteristics of unique factor H binding protein variants. GGGSGG GGGSGG GGGSGG CCCSCS GSGG 99  $\Theta\Theta$ عادادا Ü <u>ت</u> Ç Ü G 0000 ٩ ٣ |&|&|& ٣ N M M N  $\mathbf{\underline{\alpha}}$ Ą, ¥ 104 105 107 108 109 110 119 120 | 123 | 123 | 124 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 126 128 129 130 131 133 138 139 144 145 150 151 121

Ea55 Ea38  $E\alpha 2$  $E\alpha 1$ Ea11 HB99 EB4 **E**β2 Da3 Da2 Da17 Dαθ  $D\alpha4$ Da1 Da2 Da1 **D**β2 Dal  $D\alpha 1$ Dal DB1 DB1 Dα1 Dα1 Dal Da7 Dal  $D\alpha 1$ Ср10 CB15 CB10 СВ16 СВ5 Ca46 CB18 **CB15** Ca47 Ca48 CB1 CB2 Ca2 Ca6 CB) CB4  $C\alpha 1$ Ca2 **CB21** 물물물물 CB1 Bal Bal Bal Bal Вβ1  $\mathbf{B}\alpha 1$  $B\beta1$ Bal Bal Bal Bal Αα36  $A\alpha 29$ Αβ15 

 Aα7

 Aα42

 Aα43

 Aα2

 Aα7

 Aβ1

 Aα1

 Αβ11  $A\alpha 7$ AB7 Αβ5 АВ1 АВ1 АВ2 АВ2 Αβ7 Αβ1 Αβ6 Λβ3 Αβ4 Αβ4  $A\beta8$  $A\beta I$ Fig. 10E: Table 9, Characteristics of unique factor II binding protein variants. 99989999 6 GGGSGGG GGGSGG GGGSGG GGGSGG 998999 CCCSCC GGGSGG GGGSGG 998999 CCCSCC GGGSGG 666866 GGGSGG SGSGG GGSGG GGGS SGG <u>ت</u> Ġ |v|v 5 Ü Ċ C C Ü ٣ > M П П  $|\Box|$  $|\Box|$ П |== |= |□ |४|>|> > Ā Ą  $\mathbf{m} \mid \mathbf{m}$ A ¥ A A AA <¥ ¥ V A A A A A ⋖ A ¥ 3 170 155 156 157 157 165 168 169 172 158 99 163 164 166 174 175 176 178 |21 |82 |83 167 191 162 181

Eβ13 Еβ14 Еβ11  $E\alpha \theta$  $E\beta3$  $E\beta3$ Eβ3 EB3 EB3 EB3 EB3 EB3 EB3  $E\beta7$ Еβ1 Eß1 ЕВІ  $E\beta1$ Eß1 EB1  $E\beta 1$ Eß1  $\mathrm{D}\alpha 1$ Da1 Da1  $D\alpha 1$  $\frac{\mathrm{D}\alpha 1}{\mathrm{D}\alpha 1}$ Da1  $D\alpha 1$  $D\alpha 1$ Dal Dal | Dat  $\mathrm{D}\alpha\mathrm{I}$ Dal CB13 CB14 CB19 \$\frac{12}{2} \frac{12}{2} \fra CB2 CB3 CB2 CB3 СВЗ  $C\alpha 4$ Ba3 Ba2 Ba3 $B\alpha 1$  $B\alpha 1$ Bal Bα1 Bal Bal  $\mathbf{B}\alpha 1$ Bal Bal Bal  $\mathbf{B}$ Bα1  $\mathbf{B}\alpha 1$ Bal Aβ12 Aa23 Aa39 Aa36 Aa22 Aα45 Aβ3 AB13 Aa27  $A\beta4$  $A\beta8$ Aa21  $A\alpha9$  $A\alpha4$  $A\beta2$ Aβ1 Aβ1  $A\alpha 1$ Aα1 Aα1 Aα1 A<sub>α</sub>1  $\Lambda \alpha 2$  $\Lambda\beta3$ Fig. 10F: Table 9, Characteristics of unique factor H binding protein variants. GGGSGGG GGGSGG GGGSGG GGGSGG 998999 GGGSGS GGGSGS GGGSGS SGSGG GG 9999 GG 0 0 Ü ත ත G Φ G G G Ç 0 Ġ 5  $\geq$ > V V ¥ A A A K A ₹ A  $\mathbf{\alpha}$ 182 183 184 185 185 185 186 194 195 196 197 203 204 205 207 208 209 210 | 198 | 199 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 | 198 183 190 192 193 191

 $E\alpha 43$  $E\alpha 13$ Eα46 Fa42  $E\alpha 45$  $E\alpha 48$  $E\alpha 13$ Ea13  $E_{\alpha}20$  $E\alpha 20$ Ea39  $E\alpha 30$  $E\alpha 56$  $E\alpha 52$ **En36**  $E\alpha 32$ **E**a44  $E\alpha 47$  $E\alpha 37$  $E\alpha 31$  $E\alpha 2$  $\mathrm{E}\mathfrak{a}8$ Ea3 $E\alpha51$ Ea3 $E_{0}2$ Ea11  $E\alpha 8$  $D\alpha 15$  $D\alpha 10$ Dall Da3 $D\alpha\beta$  $D\alpha\beta$ Da2 Da2 Da2 Da5 Da5 Das Das Da4  $D\alpha 1$  $D\alpha 1$  $D\alpha 1$  $D\alpha 1$  $D\alpha 1$  $D\alpha 1$ Dal  $D\alpha 1$ Dail  $D\alpha 1$ Dal  $D\alpha 1$ Da1 Dal Da1 Da1 Ca36 Ca36 Ca36 Ca19  $C\alpha 19$ Ca39 Ca36 Ca37 Ca34 Ca33 Ca40 Ca23 Ca25 Ca24 Ca3 $C\alpha 27$ Ca5Ca6 Ca3 Ca3 Ca3 Ca3 Ca2 Ca2 Cal Cal Ca1 $C\alpha 1$ Cal Ba6 Ba6 Ba9  $B\alpha 2$  $B\alpha 1$ Ba7 Bal Bal Bal  $B\alpha I$  $B\alpha 1$ Bal  $B\alpha \mathbf{1}$ Ba1  $B\alpha \mathbf{l}$ Bα1  $B\alpha 1$ Bal  $B\alpha 1$  $B\alpha 1$ Bα1 Bal  $\mathbf{B}$ Aa12 AB10 Αα29 Aa32  $A\alpha 32$ Αα2 Λα33 Aa36 Aa34 Aa24Aa31 Aa31 Aa27 Aa11  $A\alpha 2$ A<sub>0</sub>2 Aa3  $A\alpha 2$ Aa2  $A\alpha 1$  $A\alpha 1$  $A\alpha 1$ Λα1  $A\alpha 1$ Aαl Fig. 10G: Table 9, Characteristics of unique factor H binding protein variants. GGGSGG 99S999 998999 998999 CCCSCC 998999 GGGSGG GGGSGG GGGSGG GGSGG G G Ü Ü G G C ٣ G G  ${\bf G}$ 000 00  $\geq$  $\geq$ В മിമിമ  $\mathbf{m}$ B B m  $\square$ B  $\mathbf{m}$ mm 214 215 217 236 237 238 239 240 241

EPL Ea16 Ea65 Ea10 Ha10 Ea53 Ea23 Ea18  $R\alpha 55$ Εα54 E033 Ea38 Ea40  $E\alpha 11$ Ea6 Eas Eas Ea6 Ea3 Ea6  $E\alpha \theta$  $E\alpha41$ Ea5 E<sub>α</sub>1 Ea6 Da12 Da13 Da5 Da2 Da2 Da4 Da6 Da2  $D\alpha 5$ Da4 Dal Dal Dal  $D\alpha 1$ Dα1 Dal Da1 Dal Dal Dal Da1 Dal Dal Dal  $\mathrm{D}\alpha 1$ Dal Ca42 Ca44 Ca35 Ca19 Ca43 Ca10Ca28 Ca33 Ca32  $C\alpha 30$ Ca29 Ca29  $C\alpha 29$ CB22 Ca5Ca41 Ca1 Ca6 Ca27 Ca2 Ca2 Ca2 Ca31 Co2 Ca2  $C\alpha 5$ CaS  $C\alpha 5$ Ca2Ca1Bal Bal Ba4 Ba5 Bo2 Bo2 Ba2  $\mathbf{B}$ Bα1  $\mathbf{B}\alpha2$ Βαl  $\mathbf{B}\alpha 1$  $\mathbf{B}\alpha 1$  $\mathbf{B}\alpha\mathbf{I}$  $\mathbf{B}\alpha\mathbf{1}$  $\mathbf{B}\alpha 1$ Bαl Bα1  $\mathbf{B}\alpha\mathbf{I}$ Bal Bal Aα2 Λα35 Aa18 Aa12 Αα19 Aa25 Aa19 Αα20 A<sub>0</sub>29 Aa26 Aa28 Aa30 Αα2 Αα2 Αα2 Aa2 Aa2 Aa2 Aa1  $A\alpha 7$  $A\alpha 2$ Au3 Aa3 AB7 Fig. 10H: Table 9, Characteristics of unique factor H binding protein variants. CCCSCC GGGSGG GGGSGG 998999 GGGSGG 666866 0000 0|0|0 0 0 0 0 Ç 0 G 0 0 В 

Fig. 101: Table 9, Characteristics of unique factor H binding protein variants.

	EB15	Eα46
	DB1	$D\alpha 1$
	CB1	Ca6
	$B\alpha 1$	$B\alpha 1$
	Aa1	Aa7
Property Common	G	GGGSGG
i	III	I
men or analas tasto	A	В
١.	2	1
The second of the second	274	275

Protein IDs were obtained from the Neisseria org fHbp peptide database.

<sup>2</sup>Variant groups as described by Masignani et al (2003) J Exp Med 197(6):789-99

<sup>3</sup>Sub-family Å or B as described by Fietcher et al (2004) *Infect Immun* 72(4):2088-100 and Murphy et al (2009) *J Infect Dis* <sup>4</sup>Modular classification according to the present disclosure.

<sup>5</sup>Segment alleles as described by Beernink and Granoff (2009) *Microbiology* 155(Pt 9):2873-83. See also Table 5 <sup>6</sup>Two protein variants had three additional amino acids at their C-termini (See text) <sup>7</sup>NA, not available

Fig. 11

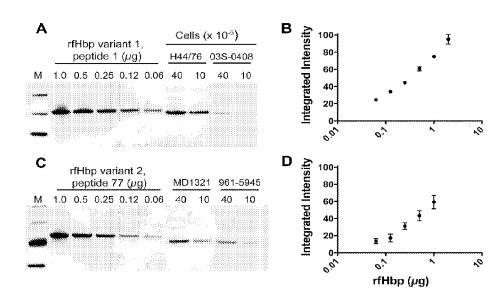


Fig. 12

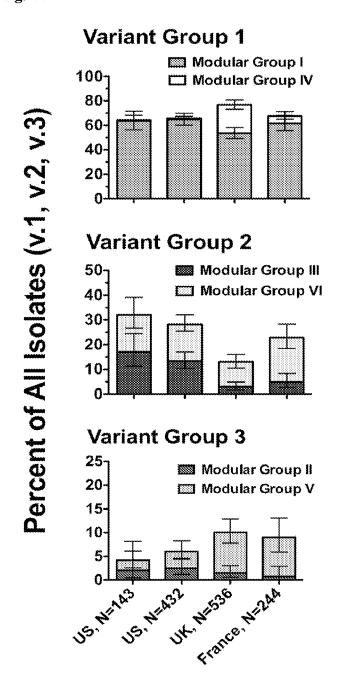
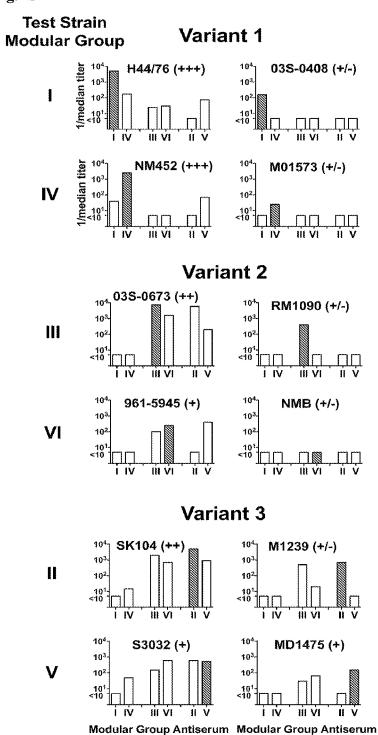


Fig. 13



# CHIMERIC FACTOR H BINDING PROTEINS (FHBP) AND METHODS OF USE

# CROSS REFERENCED TO RELATED APPLICATION

This application claims priority to U.S. Provisional Application No. 61/174,424 filed Apr. 30, 2009, which is incorporated herein by reference in its entirety and for all purposes.

# STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

This invention was made with government support under National Institutes of Health grant nos. R01 Al46464 and C06 RR16226. The government has certain rights in this invention.

## FIELD OF THE INVENTION

This invention relates to vaccines for diseases caused by *Neisseria meningitidis*.

#### INTRODUCTION

Neisseria meningitidis is a Gram-negative bacterium which colonizes the human upper respiratory tract and is responsible for worldwide sporadic and cyclical epidemic outbreaks of, most notably, meningitis and sepsis. The attack and morbidity rates are highest in children under 2 years of 30 age. Like other Gram-negative bacteria, Neisseria meningitidis typically possess a cytoplasmic membrane, a peptidoglycan layer, an outer membrane which together with the capsular polysaccharide constitute the bacterial wall, and pili, which project into the outside environment. Encapsulated 35 strains of Neisseria meningitidis are a major cause of bacterial meningitis and septicemia in children and young adults. The prevalence and economic importance of invasive Neisseria meningitidis infections have driven the search for effective vaccines that can confer immunity across different 40 strains, and particularly across genetically diverse group B strains with different serotypes or serosubtypes.

Factor H Binding Protein (fHbp, also referred to in the art as lipoprotein 2086 (Fletcher et al, Infect Immun 2004; 72:2088-2100), Genome-derived *Neisserial* antigen (GNA) 45 1870 (Masignani et al. J Exp Med 2003; 197:789-99) or "741") is an *N. meningitidis* protein which is expressed in the bacterium as a surface-exposed lipoprotein. An important function of fHbp is to bind human complement fH, which down-regulates complement activation. Binding of fH to the 50 bacterial surface is an important mechanism by which the pathogen survives in non-immune human serum or blood and evades innate host defenses.

From analysis of 71 *N. meningitidis* strains in the first study, and more than 200 strains in the second study, representative of its genetic and geographic diversity, *N. meningitidis* strains have been sub-divided into three fHbp variant groups (referred to as variant 1 (v.1), variant 2 (v.2), and variant 3 (v.3)) based on amino acid sequence variability and immunologic cross-reactivity (Masignani et al. J Exp Med 60 2003; 197:789-99). Other workers (Fletcher et al, 2004) have subdivided the protein into two sub-families designated A (which includes v.2 and v.3 of Masignani) and B (v.1). Variant 1 strains account for about 60% of disease-producing group B isolates (Masignani et al. 2003, supra). Within each variant 6 group, there is on the order of about 92% or greater conservation of amino acid sequence. Specifically, conservation

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within each variant group ranges between 89 and 100%, while between the variant groups (e.g., between v.1 and v.2) the conservation can be as low as 59%. The protein is expressed by all known strains of *N. meningitidis*.

There remains a need for a single fHbp polypeptide that can elicit bactericidal antibody responses that are effective against a broad spectrum of strains expressing different fHbp variants.

#### SUMMARY

Chimeric fHbps that can elicit antibodies that are bactericidal for different fHbp variant strains of *N. meningitidis*, and methods of use, are provided.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A-1G is Table 7 that lists the source strains and characteristics of about 69 unique factor H binding proteins.

FIG. **2A-2**E is Table 8 that lists unique sequences in fHbp variable segment A through E and number of unique peptides from each variant group containing the segment ID. Segment ID is an identifier for each variable segment. Distinct sequence variants were assigned a unique identifier beginning with a letter, A through E, to represent the segment; followed by an  $\alpha$  or  $\beta$  to indicate homology of the segment with either the corresponding fHbp segment from peptide ID 1 (fHbp v.1) or peptide ID 28 (fHbp v.3), respectively, followed by a number for each distinct sequence. Numbers in variant groups 1, 2, and 3 are provided for some segment IDs of each segment (A through E) in subtables.

FIG. 3 is a phylogram of fHbp based on 69 unique amino acid sequences.

FIG. 4, panel A is a schematic representation of fHbp showing positions of linkers, which are also referred herein as invariant segment (I), shown as vertical rectangles, together with variable segments (V). Panel B is a table listing the amino acid sequence for each invariant segment.

FIG. 5 is a phylogram of unique fHbp amino acid sequences in variable segments A (residues 8-73) (panel A) and B (residues 79-93) (panel B).

FIG. 6 is a phylogram of unique fHbp amino acid sequences in variable segments C (residues 98-159) (panel A) and D (residues 162-180) (panel B).

FIG. 7 is a phylogram of unique fHbp amino acid sequences in variable segment E (residues 186-253).

FIG. **8**, panel A is a schematic representation of 9 fHbp modular architectures deduced from phylogenic analysis, including the more common 6 modular group types. Gray segments represent  $\alpha$  progenitor sequences homologous to peptide ID 1 while white segments represent  $\beta$  progenitor sequences homologous to peptide 28. About 94% of the unique fHbp amino acid sequences analyzed herein belong to one of the first six modular group types (I, II, III, IV, V, and VI). Panel B, Four of the analyzed fHbps are classified as one of three modular architectures shown. The architectures contain junction points within a variable segment, designated by arrows. Panel C is a table listing the amino acid residues at which an  $\alpha$  progenitor sequence switches to a  $\beta$  or vice versa (J<sub>1</sub>, J<sub>2</sub>, and J<sub>3</sub>) within a variable segment.

FIG. 9 presents structural models of factor H binding protein based on the coordinates of fHbp in a complex with a fragment of human factor H (Schneider et al. (2009) *Nature* 458:890-3). Left models in each of the following panels show fHbps facing membrane side; center models are facing exposed side; and the right models depict membrane side of the fHbp down. Panel A, Cartoon representation depicting the

two structural domains. B, Space-filling model with factor H-binding residues depicted in black and the amino acid residues of the invariant segments in white. C, Space-filling model with the amino acid residues of the invariant segments depicted in white as in panel B, and the residues affecting the 5 epitopes of anti-fHbp mAbs shown in black.

FIG. 10A-10I is Table 9 that lists certain characteristics of unique factor H binding protein variants use in an analysis that include 275 peptide IDs. Sequence identifiers for N-terminal variable sequences are as follows: GGGS (SEQ ID NO: 10 7), SGSGG (SEQ ID NO: 8), GGGSGG (SEQ ID NO:9), GGGSGS (SEQ ID NO:10), GSGG (SEQ ID NO:11), GGGSGGGG (SEQ ID NO:12), GGGSGGGSGG (SEQ ID NO:13), and GGSGG (SEQ ID NO:14).

FIG. 11 shows fHbp expression measured by immunoblot- 15 ting with infrared detection. Panels A and C, Recombinant proteins in modular groups I (ID 1) or VI (ID 77), or heatkilled bacterial cells from strains expressing fHbp in the corresponding modular groups. The modular group I proteins were detected with murine anti-fHbp mAb JAR 5, which 20 recognizes nearly all fHbps in modular groups I and IV. The modular group VI proteins were detected with anti-fHbp mAb JAR 31, which recognizes nearly all proteins in modular groups II, III, V and VI (See Table 6 in example section). Panels B and D, Standard curves from the corresponding 25 elicit antibodies that are bactericidal for different fHBP varibinding of the recombinant proteins shown in Panels A and C.

FIG. 12 shows the frequencies of fHbp modular groups among systematically collected N. meningitidis group B case isolates. Data are from sequences of isolates collected in the United States (N=432), United Kingdom (N=536) and France 30 (N=244) reported by Murphy et al [16], and newly obtained sequences of 143 additional U.S. isolates from California (2003-2004), Maryland (1995 and 2005), and pediatric hospitals in 9 states (2001-2005).

FIG. 13 shows serum bactericidal activities of serum pools 35 from mice immunized with fHbps from modular groups I to VI. The black bars represent the median titers of 3 to 4 serum pools for each modular group tested against homologous test strains. The white bars represent the median titer of the respective heterologous serum pools against the test strain. +, 40 refers to relative expression of fHbp by each of the strains; strains with +/- representing low fHbp-expressing strains (see values in Table 5 in example section)

Before the present invention and specific exemplary embodiments of the invention are described, it is to be under- 45 stood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or 55 intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated 60 range includes one or both of the limits, ranges excluding either both of those included limits are also included in the invention.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood 65 by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or

equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

It must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an antigen" includes a plurality of such antigens and reference to "the protein" includes reference to one or more proteins, and so forth.

The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

## DETAILED DESCRIPTION OF EXEMPLARY EMBODIMENTS OF THE INVENTION

The present disclosure provides chimeric fHbps that can ant strains of N. meningitidis, and methods of use.

#### **DEFINITIONS**

"Factor H Binding Protein" (fHbp), which is also known in the literature as GNA1870, GNA 1870, ORF2086, LP2086 (lipoprotein 2086), and "741" refers to a polypeptide of N. meningitidis that is a lipoprotein presented on the surface of the bacterium. N. meningitidis strains have been sub-divided into three fHbp variant groups (referred to as variant 1 (v.1), variant 2 (v.2), and variant 3 (v.3) in some reports (Masignani et al. 2003, supra) and Family A and B in other reports (see, e.g., Fletcher et al. 2004 Infect Immun 2088-2100)) based on amino acid sequence variability and immunologic cross-reactivity (Masignani et al. JExp Med 2003; 197:789-99). Each unique fHbp found in N. meningitidis is also assigned a peptide ID according to neisseria.org, as shown in FIGS. 1A-1G (Table 7) and FIGS. 10A-10I (Table 9). For clarity, the present disclosure uses the v.1, v.2 and v.3 terminology. Because the length of variant 2 (v.2) fHbp protein (from strain 8047, peptide ID 77) and variant 3 (v.3) fHBP (from strain M1239, peptide ID 28) differ by -1 and +7 amino acid residues, respectively, from that of MC58 (peptide ID 1), the numbering used to refer to residues for v.2 and v.3 fHbp proteins differs from numbering based on the actual amino acid sequences of these proteins. Thus, for example, reference to a leucine residue (L) at position 166 of the v.2 or v.3 fHBP sequence refers to the residue at position 165 of the v.2 protein and at position 173 in the v.3 protein.

The term "heterologous" or "chimeric" refers to two components that are defined by structures derived from different sources. For example, where "heterologous" is used in the context of a chimeric polypeptide, the chimeric polypeptide includes operably linked amino acid sequences that can be derived from different polypeptides of different phylogenic groupings (e.g., a first component from an  $\alpha$  and a second component from a β progenitor amino acid sequences). Similarly, "heterologous" in the context of a polynucleotide encoding a chimeric polypeptide includes operably linked nucleic acid sequence that can be derived from different genes (e.g., a first component from a nucleic acid encoding a fHbp v.1 polypeptide and a second component from a nucleic acid

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encoding a fHbp v.2 polypeptide). Such chimeric polypeptides as described herein provide for presentation of epitopes in a single polypeptide that are normally found in different polypeptides. Other exemplary "heterologous" nucleic acids include expression constructs in which a nucleic acid com- 5 prising a coding sequence is operably linked to a regulatory element (e.g., a promoter) that is from a genetic origin different from that of the coding sequence (e.g., to provide for expression in a host cell of interest, which may be of different genetic origin relative to the promoter, the coding sequence or 10 both). For example, a T7 promoter operably linked to a polynucleotide encoding an fHbp polypeptide or domain thereof is said to be a heterologous nucleic acid. "Heterologous" in the context of recombinant cells can refer to the presence of a nucleic acid (or gene product, such as a polypeptide) that is of 15 a different genetic origin than the host cell in which it is present. For example, a Neisserial amino acid or nucleic acid sequence of one strain is heterologous to a Neisserial host of another strain.

"Derived from" in the context of an amino acid sequence or polynucleotide sequence (e.g., an amino acid sequence "derived from" a v.1 fHbp) is meant to indicate that the polypeptide or nucleic acid has a sequence that is based on that of a reference polypeptide or nucleic acid (e.g., a naturally occurring fHbp protein or encoding nucleic acid), and is 25 not meant to be limiting as to the source or method in which the protein or nucleic acid is made. "Derived from" in the context of bacterial strains is meant to indicate that a strain was obtained through passage in vivo, or in in vitro culture, of a parental strain and/or is a recombinant cell obtained by 30 modification of a parental strain.

"Conservative amino acid substitution" refers to a substitution of one amino acid residue for another sharing chemical and physical properties of the amino acid side chain (e.g., charge, size, hydrophobicity/hydrophilicity). "Conservative 35 substitutions" are intended to include substitution within the following groups of amino acid residues: gly, ala; val, ile, leu; asp, glu; asn, gln; ser, thr; lys, arg; and phe, tyr. Conservative amino acid substitutions in the context of a chimeric fHBP disclosed herein are selected so as to preserve presentation of 40 an epitope of interest. Guidance for such substitutions can be drawn from alignments of amino acid sequences of polypeptides presenting the epitope of interest.

The term "protective immunity" means that a vaccine or immunization schedule that is administered to a mammal 45 induces an immune response that prevents, retards the development of, or reduces the severity of a disease that is caused by *Neisseria meningitidis*, or diminishes or altogether eliminates the symptoms of the disease. Protective immunity can be accompanied by production of bactericidal antibodies. It should be noted that production of bactericidal antibodies against *Neisseria meningitidis* is accepted in the field as predictive of a vaccine's protective effect in humans. (Gold-schneider et al., 1969, J. Exp. Med. 129:1307; Borrow et al. 2001 Infect Immun. 69:1568).

The phrase "a disease caused by a strain of capsular group B of *Neisseria meningitidis*" encompasses any clinical symptom or combination of clinical symptoms that are present in an infection of a human with a member of capsular group B of *Neisseria meningitidis*. These symptoms include but are not 60 limited to: colonization of the upper respiratory tract (e.g. mucosa of the nasopharynx and tonsils) by a pathogenic strain of capsular group B of *Neisseria meningitidis*, penetration of the bacteria into the mucosa and the submucosal vascular bed, septicemia, septic shock, inflammation, haemmorrhagic skin lesions, activation of fibrinolysis and of blood coagulation, organ dysfunction such as kidney, lung, and

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cardiac failure, adrenal hemorrhaging and muscular infarction, capillary leakage, edema, peripheral limb ischaemia, respiratory distress syndrome, pericarditis and meningitis.

The phrase "broad spectrum protective immunity" means that a vaccine or immunization schedule elicits "protective immunity" against at least more than one strain (and can be against at least two, at least three, at least four, at least five, against at least eight, or more strains) of *Neisseria meningitidis*, wherein each of the strains expresses a different fHbp subvariant or fHbp variant. The present disclosure specifically contemplates and encompasses a vaccine or vaccination regimen that confers protection against a disease caused by a member of any capsular group (e.g., A, B, or C), with protection against disease caused by a capsular group B strain of *Neisseria meningitidis* being of interest due to the epidemiological prevalence of strains causing disease with this capsular group and lack of broadly effective group B vaccines.

The phrase "specifically binds to an antibody" or "specifically immunoreactive with", in the context of an antigen (e.g., a polypeptide antigen) refers to a binding reaction which is based on and/or is probative of the presence of the antigen in a sample which may also include a heterogeneous population of other molecules. Thus, under designated conditions, the specified antibody or antibodies bind(s) to a particular antigen or antigens in a sample and do not bind in a significant amount to other molecules present in the sample. "Specifically binds to an antibody" or "specifically immunoreactive with" in the context of an epitope of an antigen (e.g., an epitope of a polypeptide) refers to a binding reaction which is based on and/or is probative of the presence of the epitope in an antigen (e.g., polypeptide) which may also include a heterogeneous population of other epitopes, as well as a heterogeneous population of antigens. Thus, under designated conditions, the specified antibody or antibodies bind(s) to a particular epitope of an antigen and do not bind in a significant amount to other epitopes present in the antigen and/or in the sample.

amino acid substitutions in the context of a chimeric fHBP disclosed herein are selected so as to preserve presentation of an epitope of interest. Guidance for such substitutions can be drawn from alignments of amino acid sequences of polypeptides presenting the epitope of interest.

The term "protective immunity" means that a vaccine or immunization schedule that is administered to a mammal induces an immune response that prevents, retards the development of, or reduces the severity of a disease that is caused by *Neisseria meningitidis*, or diminishes or altogether elimi-

A "surface antigen" is an antigen that is present in a surface structure of *Neisseria meningitidis* (e.g. the outer membrane, capsule, pili, etc.).

"Isolated" refers to an entity of interest that is in an environment different from that in which the compound may naturally occur. "Isolated" is meant to include compounds that are within samples that are substantially enriched for the compound of interest and/or in which the compound of interest is partially or substantially purified."

"Enriched" means that a sample is non-naturally manipulated (e.g., by an experimentalist or a clinician) so that a compound of interest is present in a greater concentration (e.g., at least a three-fold greater, at least 4-fold greater, at least 8-fold greater, at least 64-fold greater, or more) than the concentration of the compound in the starting sample, such as a biological sample (e.g., a sample in which the compound naturally occurs or in which it is present after administration), or in which the compound was made (e.g., as in a bacterial polypeptide, antibody, chimeric polypeptide, and the like)

A "knock-out" or "knockout" of a target gene refers to an alteration in the sequence of the gene that results in a decrease of function of the target gene, e.g., such that target gene expression is undetectable or insignificant, and/or the gene product is not functional or not significantly functional. For 5 example, a "knockout" of a gene involved in LPS synthesis indicates means that function of the gene has been substantially decreased so that the expression of the gene is not detectable or only present at insignificant levels and/or a biological activity of the gene product (e.g., an enzymatic 10 activity) is significantly reduced relative to prior to the modification or is not detectable. "Knock-outs" encompass conditional knock-outs, where alteration of the target gene can occur upon, for example, exposure to a predefined set of conditions (e.g., temperature, osmolarity, exposure to sub- 15 stance that promotes target gene alteration, and the like. A "knock-in" or "knockin" of a target gene refers to a genetic alteration in a host cell genome that that results in an increase in a function provided by the target gene.

"Non-naturally occurring", as used herein, refers to a pro- 20 tein (e.g. fHbp) that is not normally found in nature and is instead artificially produced and/or modified by a human. A non-naturally occurring subject fHbp can be made via chemical synthesis or recombinant methods. For example, "nonras" and encompass fHbp with heterologous components that are not found in nature.

fHbp and fHbp-Encoding Nucleic Acids

Before describing further exemplary chimeric fHbps contemplated by the present disclosure, it is helpful to describe 30 naturally-occurring fHbps.

For convenience and clarity, the native amino acid sequence of the v.1 fHBP of the N. meningitidis strain MC58 (peptide ID 1) was arbitrarily selected as a reference sequence for all native v.1, v.2, and v.3 fHbp amino acid sequences, as 35 well as for the chimeric fHbps described herein. Unless otherwise noted, amino acid residue positions are also referred herein with reference to peptide ID 1. The sequence of peptide ID 1 is presented below:

sequences (I) and the variable segments are designated as the N-terminal element,  $V_A$ ,  $V_B$ ,  $V_C$ ,  $V_D$ , or  $V_E$  segments. The amino acid sequence of the conserved sequences are listed in FIG. 4, panel B.

As such, naturally occurring fHbps can be defined by the formula:  $I_1$ -Nte- $I_2$ - $V_A$ - $I_3$ - $V_B$ - $I_4$ - $V_C$ - $I_5$ - $V_D$ - $I_6$ - $V_E$ - $I_7$ , wherein "Nte" refers to the N-terminal element, "I" refers to an "invariant" segment, and "V" refers to a variable segment of either an  $\alpha$  or a  $\beta$  progenitor type.

As disclosed below in more detail, the non-naturally occurring chimeric fHbps of the present disclosure can be constructed by various combinations of the V segments which are flanked by I segments. The invariant and variable segments which can be used in construction of such chimeric fHbps are described from N-terminus to C-terminus in more detail below. This modular classification can also be found on the Neisseria.org website (neisseria.org/perl/agdbnet/ agdbnet.pl?file=nm\_fhbp.xml). On the website the progenitor groups  $\alpha$  and  $\beta$  as described below are referred to 1 and 2, respectively. For example,  $V_4\alpha 2-V_B\alpha 1-V_C\beta 5-V_D\alpha 5-V_E\alpha 8$ becomes A1.2-B1.1-C2.5-D1.5-E1.8.

**Invariant Segments** 

fHbps contain amino acid sequences that are conserved naturally occurring chimeras" refers to "man-made chime- 25 among all fHbp amino acid sequences analyzed herein. The conserved sequences flank variable segments that are derived from either the  $\alpha$  or  $\beta$  progenitor amino acid sequences. These conserved sequences are referred to herein as "invariant sequences" or "invariant segments", and may serve as sites of recombination in naturally-occurring fHbps. Where these invariant segments are flanked by variable segments, the invariant segments may be referred to as "linker" sequences. While these sequences exhibit amino acid sequence identity between the fHbps disclosed herein, such amino acid sequences may still tolerate certain conservative amino acid substitutions. As such, the term "invariant segment" as used herein should not be construed to be limited to the specific amino acid sequences described herein, and may at least contain one or more conservative amino acid substitutions.

(SEQ ID NO: 1)

CSSGGGGVAADIGAGLADALTAPLDHKDKGLQSLTLDQSVRKNEKLKLAAQGAEKT

YGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQVYKQSHSALTAFQTEQIQDSEHSGKMVA

KROFRIGDIAGEHTSFDKLPEGGRATYRGTAFGSDDAGGKLTYTIDFAAKOGNGKIEHLKSPELNVDLA

AADIKPDGKRHAVISGSVLYNQAEKGSYSLGIFGGKAQEVAGSAEVKTVNGIRHIGLAAKQ

Despite the diversity of variant groups and other classification schemes for the sequences listed in FIGS. 1A-1G (Table 7) and FIGS. 10A-10I (Table 9), a first analysis of 69 unique fHbp sequences and a second analysis of all 275 peptide IDs, as described in the Examples below revealed that several different stretches of amino acid sequences within the 55 fHbps are conserved among all the fHbps analyzed. As shown by the phylogenic studies presented in the Examples section below, these conserved amino acid sequences separate fHbp into modular segments of amino acid sequences that cluster in the phylogram to define two progenitor sequences from 60 which each variable segment may be derived. As illustrated in FIGS. 5-7, each of the variable segments in fHbps can be classified as being of one of the two progenitor amino acid sequences: designated for convenience,  $\alpha$  or  $\beta$ .

The nomenclature architecture used to characterize fHbp 65 based on modular segments is illustrated in FIG. 4, panel A. The rectangles in FIG. 4, panel A represent the conserved

As illustrated in FIG. 4, the most N-terminal invariant segment found in naturally-occurring fHbps starts at residue 1 and is of the amino acid sequence CSSG (SEQ ID NO:1). Immediately C-terminal to the first invariant segment CSSG  $(SEQ ID NO:2) (I_1)$  is a variable N-terminal element of about 1 to about 6 glycine or serine residues. The various N-terminal elements are listed in FIG. 1A-1G (Table 7) and FIGS. 10A-10I (Table 9). The second invariant segment  $(I_2)$  is of the amino acid sequence GG, which is positioned C-terminal to the N-terminal element and starts at residue 6, 7, or 11, depending on the length of the N-terminal element (e.g., in peptide ID 28 of variant 3, I, starts at residue 11). The next invariant segment, I<sub>3</sub>, is defined by the amino acid sequence of SRFDF (SEQ ID NO:3) and is positioned at residue 74, C-terminal to I<sub>2</sub> and a variable segment. I<sub>4</sub>, which is C-terminal to I<sub>3</sub>, is defined by the amino acid sequence GEFQ (SEQ ID NO:4) and is positioned at residue 94. I<sub>5</sub> is defined by the amino acid sequence DD and is C-terminal to I4 at residue

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159.  $I_6$ , positioned C-terminal to  $I_5$ , is defined by IEHLK (SEQ ID NO:5) or IEHLE (SEQ ID NO: 6) and starts at residue 180. Lastly,  $I_7$ , the most C-terminal invariant segment in naturally-occurring fHbps, starts at residue 252 and is defined by the amino acid sequence of KQ.

The residue position listed above at which each invariant segment starts may be shifted from 1 to 8 residues depending on the length of the N-terminal element and the amino acid sequence of the variable segments. As noted above, for convenience and clarity residue numbering used in reference to non-naturally occurring chimeric fHbps throughout the present disclosure is based on the amino acid sequence numbering of the fHbp of MC58 (peptide ID 1), which is a variant 1 strain.

Based on the sequences analyzed herein, the length of the invariant segments ranges from about 2 amino acid residues to about 5 amino acid residues.

Variable Segments

As noted above, variable segments (V) are flanked by link- 20 ers, also referred herein as invariant segments (I). In addition to a variable N-terminal element, there are five variable segments in an fHbp and they are designated from N-terminus to C-terminus as  $V_A$ ,  $V_B$ ,  $V_C$ ,  $V_D$ , and  $V_E$ , each flanked by the invariant segments discussed above. As mentioned previ- 25 ously, each of the variable segments may be classified as being of one of the two progenitor amino acid sequences:  $\alpha$  or  $\beta$ . For example,  $V_A \alpha$  indicates that variable segment A is derived from an  $\alpha$  progenitor amino acid sequence while  $V_{\alpha}\beta$ refers to a variable segment A derived from a β progenitor 30 amino acid sequence. In a more specific example,  $V_{A}\alpha 1$ denotes that the variable segment A is of the amino acid sequence set forth as Segment ID A.α.1 in FIG. 2A (Table 8, segment A), which is one allele of the  $\alpha$  progenitor sequences of variable segment A. As described below, the chimeric fHbp 35 of the present disclosure contain a non-naturally occurring combination of variable segments in which each segment may be independently selected from either an  $\alpha$  and a  $\beta$ progenitor type and/or a non-naturally occurring combination of any alleles of the corresponding segment shown in FIGS. 40 **2**A-**2**E, Table 8.

The  $\alpha$  and  $\beta$  progenitor variable segments are each defined by certain signature amino acid residues. In addition, the  $\alpha$  progenitor variable segments exhibit sequence similarity to fHbp of variant 1 group (e.g. peptide ID 1), while the  $\beta$  45 progenitor variable segments exhibit sequence similarity to fHbp of variant 3 group (e.g. peptide ID 28). Signature amino acid residues of  $\alpha$  or  $\beta$  progenitors for each variable segment are be discussed below.

In addition to being defined by their corresponding amino 50 acid signature sequence, variable segments of the same progenitor type share amino acid sequence identity of at least 80%, at least 85%, at least 90%, at least 95%, and can be 100%. When amino acid sequences of the same variable segments of different progenitor types are compared, the 55 amino acid sequence identity drops by at least 10% to 40%.

See Table 4 in Example section below for amino acid identities within and between sequence groups by segment.

Various alleles for each variable segment and their sequences are presented in FIG. **2**A-**2**E (Table 8) and are 60 described in detail below.

The most N-terminal variable segment is referred to herein as the N-terminal element (Nte) (FIG. 4). The various N-terminal elements found in the fHbps analyzed are listed in FIGS. 1A-1G (Table 7) and also in FIGS. 10A-10I (Table 9). They range in length from 1 to 6 residues of glycine or a combination of glycine and serine.

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The variable segment  $V_A$  starts at residue 7, C-terminal to  $I_2$ , and ends at residue 73, N-terminal to  $I_3$ .  $V_A$  of an  $\alpha$  progenitor ( $V_A\alpha$ ) comprises an amino acid sequence that is about 89 to 100% identical to Segment ID A. $\alpha$ .1 as presented in FIG. 2A (Table 8): VAADIGAGLA DALTAPLDHK DK SLQSLTLD QSVRKNEKLK LAAQGAEKTY GNGDSLN TGKLKNDKV (SEQ ID NO:15).  $V_A\alpha$  is further characterized by signature amino acid residues QSV, bolded in sequence. Certain residues that may have alternative amino acid residues among  $V_A\alpha$  of different fHbps are underlined.

 $V_A$  of a  $\beta$  progenitor ( $V_A\beta$ ) comprises an amino acid sequence that is about 89 to 100% identical to Segment ID A. $\beta$ .1 as set forth in FIG. 2A (Table 8): VAADIGTGLA DALTAPLDHK DKGLKSLTLE DSIPQNGTLT LSAQGAEKTF KAGDKDNSLN TGKLKNDKI (SEQ ID NO:67).  $V_A\beta$  is further characterized by signature amino acid residues DSI and/or KDN, bolded in sequence. Certain residues that have alternative or deleted amino acid residues among  $V_A\beta$  of different fHbps are underlined.

The variable segment  $V_B$  starts at residue 79, and ends N-terminal to where  $I_4$  is located, at residue 93.  $V_B$  of an  $\alpha$  progenitor ( $V_B\alpha$ ) comprises an amino acid sequence that is about 80% to 100% identical to Segment IDB. $\alpha$ .1 as set forth in FIG. 2B (Table 8): IRQIEVDGQL ITLES (SEQ ID NO:85).  $V_B\alpha$  is further characterized by signature amino acid residues IRQ, bolded in the sequence. Certain residues that have alternative or deleted amino acid residues among  $V_B\alpha$  of different fHbps are underlined.

 $V_B$  of a  $\beta$  progenitor  $(V_B\beta)$  comprises an amino acid sequence that is about 100% identical to Segment ID B. $\beta$ .1 as set forth in FIG. 2B (Table 8): VQKIEVDGQT ITLAS (SEQ ID NO:95).  $V_B\beta$  is further characterized by signature amino acid residues VQK, bolded in the sequence.

The variable segment  $V_C$  starts around residue 98, C-terminal to  $I_4$  and ends at residue 158, N-terminal to  $I_5$ .  $V_C$  of an  $\alpha$  progenitor ( $V_C\alpha$ ) comprises an amino acid sequence that is about 85 to 100% identical to Segment ID C. $\alpha$ .1 as presented in FIG. 2C (Table 8): VYKQSHSALT ALQTEQVQDS EHS GKMVAKR QFRIGDIAGE HTSFDKLPEG GRATYRGTAF GS (SEQ ID NO:96).  $V_C\alpha$  is further characterized by signature amino acid residues QDS, bolded in the sequence. Certain residues that have alternative or deleted amino acid residues among  $V_C\alpha$  of different fHbps are underlined.

 $V_C$  of a β progenitor ( $V_C$ β) comprises an amino acid sequence that is about 93 to 100% identical to Segment ID C.β.1 as presented in FIG. 2C (Table 8): IYKQDHSAVV ALQIEKINNP DKIDSLINQR SFLVSGLGGE HTAFN-QLPG GKAEYHGKAF SS (SEQ ID NO:152).  $V_C$ β is further characterized by signature amino acid residues NNP, bolded in sequence. This signature is found in all of  $V_C$ β amino acid sequences. Certain residues that have alternative or deleted amino acid residues among  $V_C$ β of different fHbps are underlined.

The variable segment  $V_D$  starts at residue 161, C-terminal to  $I_5$  and ends at residue 179, N-terminal to  $I_6$ .  $V_D$  of an  $\alpha$  progenitor ( $V_D\alpha$ ) comprises an amino acid sequence that is about 89 to 100% identical to Segment ID D. $\alpha$ .1 as presented in FIG. 2D (Table 8): AGGKLTYTID FAAKQGHGK (SEQ ID NO:174).  $V_D\alpha$  is further characterized by signature amino acid residues AG or AS, of which AG is bolded in sequence. Certain residues that have alternative or deleted amino acid residues among  $V_D\alpha$  of different fHbps are underlined.

 $V_D$  of a β progenitor  $(V_D\beta)$  comprises an amino acid sequence that is about 84 to 100% identical to Segment ID D.β.1 as presented in FIG. 2D (Table 8): PNGRLHYSID FTKKQGYGR (SEQ ID NO:192).  $V_D\beta$  is further character-

ized by signature amino acid residues PN, bolded in sequence. Certain residues that have alternative or deleted amino acid residues among  $V_{\mathcal{D}}\beta$  of different fHbps are underlined.

The variable segment  $V_E$  starts around residue 185, C-terminal to  $I_6$  and ends at residue 253, N-terminal to  $I_7$ .  $V_E$  of an  $\alpha$  progenitor ( $V_E\alpha$ ) comprises an amino acid sequence that is about 86 to 100% identical to Segment ID E. $\alpha$ . 1 as presented in FIG. 2E (Table 8): SPELNVDLAA AYIKPDEKHH AVISGSVLYN QAEKGSYSLG IFGGKAQEVA GSAEV 10 KTVNG IRHIGLAA (SEQ ID NO:195).  $V_E\alpha$  is further characterized by signature amino acid residues SLGI (SEQ ID NO: 281), bolded in sequence. Certain residues that have alternative or deleted amino acid residues among  $V_E\alpha$  of different fHbps are underlined.

 $V_E$  of a  $\beta$  progenitor ( $V_E\beta$ ) comprises an amino acid sequence that is about 94 to 100% identical to Segment ID E. $\beta$ .1 as set forth in FIG. 2E (Table 8): TPEQNVELAS AELKADEKSH AVILGDTRYG GEEKGTYHLA LFGDRAQEIA GSATVKIREK VHEIGIAG (SEQ ID 20 NO:262).  $V_E\beta$  is further characterized by signature amino acid residues HLAL, bolded in sequence. Certain residues that have alternative or deleted amino acid residues among  $V_E\beta$  of different fHbps are underlined.

As noted above, for convenience and clarity residue numbering used in reference to non-naturally occurring chimeric fHbps throughout the present disclosure is based on the amino acid sequence numbering of the fHbp of MC58 (peptide ID 1). In addition, the residue position listed above at which each variable segment starts and ends may be shifted from 1 to 8 residues depending on the length of the N-terminal element and the amino acid sequence of the variable segments.

fHbp Modular Groups

Naturally occurring fHbp amino acid sequences can be 35 described according to a modular architecture based on a combination of  $\alpha$  or  $\beta$  progenitor segments flanked by invariable segments, as shown in FIG. 1A-1G (Table 7) and FIGS. 10A-10I (Table 9). As described in the Examples below, naturally occurring fHbps exhibit particular combinations of 40 modular segments, and can be classified accordingly into nine modular group types as shown in FIG. 8, where gray variable segments correspond to the  $\alpha$  progenitor sequences and white segments correspond to the  $\beta$  progenitor sequences.

The Type I modular group is characterized by variable 45 segments, all of which are derived from the  $\alpha$  progenitor amino acid sequences. FHbps classified as belonging to the Type I modular group may be represented by the following formula: I<sub>1</sub>-Nte-I<sub>2</sub>-V<sub>4</sub> $\alpha$ -I<sub>3</sub>-V<sub>B</sub> $\alpha$ -I<sub>4</sub>-V<sub>C</sub> $\alpha$ -I<sub>5</sub>-V<sub>D</sub> $\alpha$ -I<sub>6</sub>-V<sub>E</sub> $\alpha$ -I<sub>7</sub>. An example of a fHbp that is of the Type I modular group is 50 peptide ID 1 from strain MC58. As noted above, peptide ID is from the factor H-binding protein database at Neisseria.org.

The Type II modular group is characterized by variable segments, all of which are derived from the  $\beta$  progenitor. FHbps classified as belonging to the Type II modular group 55 may be represented by the following formula:  $I_1$ -Nte- $I_2$ - $V_A\beta$ - $I_3$ - $V_B\beta$ - $I_4$ - $V_C\beta$ - $I_5$ - $V_D\beta$ - $I_6$ - $V_E\beta$ - $I_7$ . An example of a fHbp that is of the Type II modular group is peptide ID 28 from strain M1239. Nearly 60% of the fHbps in FIG. 1A-1G (Table 7) belong in either Type I or II modular group.

Some fHbp, however, have variable segments from both  $\alpha$  and  $\beta$  progenitors. One modular group defined by variable segments derived from both progenitors is Type III, in which variable segments  $V_A$  and  $V_B$  are derived from the  $\alpha$  progenitor and the rest of the variable segments from the  $\beta$  progenitor. 65 FHbps classified as belonging to the Type III modular group may be represented by the following formula:  $I_1$ -Nte- $I_2$ - $V_A\alpha$ -

 $I_3$ - $V_B\alpha$ - $I_4$ - $V_C\beta$ - $I_5$ - $V_D\beta$ - $I_6$ - $V_E\beta$ - $I_7$ . One example of a fHbp that belongs to the Type III modular group is peptide ID 22 from strain RM1090.

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Another modular group defined by variable segments derived from both progenitors is Type IV. Type IV is a modular group that has a variable segment  $V_A$  derived from the  $\beta$  progenitor and the rest of the variable segments from the  $\alpha$  progenitor. FHbps classified as belonging to the Type IV modular group may be represented by the following formula:  $I_1$ -Nte- $I_2$ - $V_A\beta$ - $I_3$ - $V_B\alpha$ - $I_4$ - $V_C\alpha$ - $I_5$ - $V_D\alpha$ - $I_6$ - $V_E\alpha$ - $I_7$ . One example of a fHbp that belongs to the Type IV modular group is peptide ID 15 from strain NM452.

An additional modular group is Type V, in which the variable segment  $V_D$  is derived from the  $\alpha$  progenitor and the rest of the variable segments from the  $\beta$  progenitor. FHbps classified as belonging to the Type V modular group may be represented by the following formula:  $I_1$ -Nte- $I_2$ - $V_A\beta$ - $I_3$ - $V_B\beta$ - $I_4$ - $V_C\beta$ - $I_5$ - $V_D\alpha$ - $I_6$ - $V_E\beta$ - $I_7$ . One example of a fHbp that belongs to the Type V modular group is peptide ID 79 from strain S3032.

Another modular group set forth in FIG. **8** is Type VI, in which variable segments  $V_C$  and  $V_E$  are derived from the  $\beta$  progenitor and the rest of the variable segments from the  $\alpha$  progenitor. FHbps classified as belonging to the Type VI modular group may be represented by the following formula:  $I_1$ -Nte- $I_2$ - $V_A\alpha$ - $I_3$ - $V_B\alpha$ - $I_4$ - $V_C\beta$ - $I_5$ - $V_D\alpha$ - $I_6$ - $V_E\beta$ - $I_7$ . One example of a fHbp that belongs to the Type VI modular group is peptide ID 16 from strain 961-5945.

Another modular group set forth in FIG. **8** is Type VII, in which variable segment  $V_E$  are derived from the  $\beta$  progenitor and the rest of the variable segments from the  $\alpha$  progenitor. FHbps classified as belonging to the Type VI modular group may be represented by the following formula:  $I_1$ -Nte- $I_2$ - $V_A\alpha$ - $I_3$ - $V_B\alpha$ - $I_4$ - $V_C\alpha$ - $I_5$ - $V_D\alpha$ - $I_6$ - $V_E\beta$ - $I_7$ . One fHbp found to have the Type VI modular group is peptide ID 207 from strain 0167/03.

Another modular group set forth in FIG. **8** is Type VIII, in which variable segments  $V_B$  is derived from the  $\alpha$  progenitor and the rest of the variable segments from the  $\beta$  progenitor. FHbps classified as belonging to the Type VI modular group may be represented by the following formula:  $I_1$ -Nte- $I_2$ - $V_A\beta$ - $I_3$ - $V_B\alpha$ - $I_4$ - $V_C\beta$ - $I_5$ - $V_D\beta$ - $I_6$ - $V_E\beta$ - $I_7$ . One fHbp found to have the Type VIII modular group is peptide ID 67 from strain MA-5756.

The last modular group set forth in FIG. **8** is Type IX, in which variable segments  $V_B$  and  $V_D$  are derived from the  $\alpha$  progenitor and the rest of the variable segments from the  $\beta$  progenitor. FHbps classified as belonging to the Type VI modular group may be represented by the following formula:  $I_1$ -Nte- $I_2$ - $V_A\beta$ - $I_3$ - $V_B\alpha$ - $I_4$ - $V_C\beta$ - $I_5$ - $V_D\alpha$ - $I_6$ - $V_E\beta$ - $I_7$ . One Example of a fHbp that belongs to the Type IX modular group is peptide ID 175 from strain 19498.

More than 98% of the unique fHbp amino acid sequences analyzed herein belong to one of the first six types of modular groups (I, II, III, IV, V, and VI) described above and schematically presented in FIG. 8. The numbers of unique fHbp that belong to each of the modular groups are also listed on the right of the schematic in FIG. 8.

Four naturally occurring fHbps vary at least slightly from this nomenclature, and do not precisely fit into the first six modular group types set forth in FIG. **8**, panel A. These four fHbps "switch" from one progenitor amino acid sequence to another at a residue position that is within a canonical variable segment as described above. As a result, they each contain a 65 heterologous variable segments and their junction points deviate from junction points of the rest of the fHbps found to be at I<sub>3</sub>, I<sub>4</sub>, I<sub>5</sub>, or I<sub>6</sub> defined above. See FIG. **8**, panel B for the

modular architecture of these four fHbps along with the arrow pointing at the junction point for each. However, the junction points of these four naturally occurring fHbps still reside at conserved residue(s) found within the variable segment. See table in FIG. 8, panel C.

One such fHbp is a naturally occurring fHbp derived from strain CDC-1573 (peptide ID 55), in which the junction point where  $V_A\beta$  switches to  $V_A\alpha$  resides in the middle of  $V_A$ . The heterologous  $V_A$  of peptide ID 55 may be represented as  $V_A(\beta \rightarrow \alpha)$ , signifying that the variable segment A comprises a β progenitor amino acid sequence starting at the N-terminus and switches to an  $\alpha$  progenitor amino acid sequence toward the C-terminus. The junction point  $(J_1)$  of peptide ID 55 resides at a sequence of AQGAE (SEQ ID NO:278) conserved among all the  $V_A$  sequences, which starts at residue position 50. Two other fHbps, which contain junction points that are not in the residue position of I<sub>3</sub>, I<sub>4</sub>, I<sub>5</sub>, or I<sub>6</sub>, are peptide ID 24 and peptide ID 25, derived from strain M08 240117 and strain 6275, respectively. Their junction points (J<sub>2</sub>) at which  $V_B\alpha$  switches to  $V_B\beta$  reside in the middle of  $V_B$  (residue 82), 20 NZ98/254); AY548374 (AAS56917.1) (from N. meningitidis at which there is an amino acid sequence of IEV) conserved among all  $V_B$ s. The  $V_B$  of peptide ID 24 and 25 may be represented as  $V_B(\alpha \rightarrow \beta)$ . Lastly, peptide ID 82 from strain MD1475 switches from  $V_E \alpha$  to  $V_E \beta$  at residue A196 (J<sub>3</sub>), For purposes of identifying relevant amino acid sequences which is conserved among all  $V_E$ . The variable segment E of 25 contemplated for use in the chimeric fHbps disclosed herein, peptide ID 82 may be represented as  $V_E(\alpha \rightarrow \beta)$ .

As will be discussed in detail below, chimeric fHbps can be constructed using the invariant and variable segments of this modular architecture to produce amino acid sequences not found in nature.

Nucleic Acids Encoding fHbp Segments

fHbp polypeptides, and encoding nucleic acids, from which segments of the chimeric fHbps of the present disclosure can be derived may be from any suitable N. meningitidis strain. As is known in the art, N. meningitidis strains are 35 divided into serologic groups (capsular groups), serotypes (PorB phenotypes) and subtypes (PorA phenotypes) on the basis of reactions with polyclonal (Frasch, C. E. and Chapman, 1973, J. Infect. Dis. 127: 149-154) or monoclonal antibodies that interact with different surface antigens. Capsular 40 grouping traditionally has been based on immunologically detectable variations in the capsular polysaccharide but is being replaced by PCR of genes encoding specific enzymes responsible for the biosynthesis of the structurally different capsular polysaccharides. About 12 capsular groups (includ- 45 ing A, B, C, X, Y, Z, 29-E, and W-135) are known. Strains of the capsular groups A. B. C. Y and W-135 account for nearly all meningococcal disease. Serotyping traditionally has been based on monoclonal antibody defined antigenic differences in an outer membrane protein called Porin B (PorB). Anti- 50 bodies defining about 21 serotypes are currently known (Sacchi et al., 1998, Clin. Diag. Lab. Immunol. 5:348). Serosubtyping has been based on antibody defined antigenic variations on an outer membrane protein called Porin A (PorA). Both serotyping and serosubtyping are being 55 replaced by PCR and/or DNA sequencing for identification of genes encoding the variable regions of PorB and PorA, respectively that are associated with mAb reactivity (e.g. Sacchi, Lemos et al., supra; Urwin et al., 1998, Epidem. and Infect. 120:257).

While N. meningitidis strains of any capsular group may be used, N. meningitidis strains of capsular group B are of particular interest as sources from which nucleic acid encoding fHbp and domains thereof are derived.

While the specification provides the amino acid sequence 65 of examples of fHbps from which the chimeric fHbp can be derived, this is not intended to be limiting. For example, the

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chimeric fHbp can contain amino acid sequences that are at least 80%, at least 85%, at least 90%, or at least 95% identical to an amino acid sequence of a naturally occurring fHbp.

Nucleic acids encoding fHbp polypeptides for use in construction of chimeric fHbps contemplated herein are known in the art. Examples of fHbp polypeptides are described in, for example, WO 2004/048404; Masignani et al. 2003 J Exp Med 197:789-799; Fletcher et al. Infect Immun 2004 2088-2100; Welsch et al. J Immunol 2004 172:5606-5615; and WO 99/57280. Nucleic acid (and amino acid sequences) for fHbp variants and subvariants are also provided in GenBank as accession nos.: NC\_003112, GeneID: 904318 (NCBI Ref. NP\_274866), peptide ID 1 from N. meningitidis strain MC58; AY548371 (AAT01290.1) (from N. meningitidis strain CU385); AY548370 (AAT01289.1) (from N. meningitidis strain H44/76); AY548377 (AAS56920.1) peptide ID 4 from N. meningitidis strain M4105; AY548376 (AAS56919.1) (from N. meningitidis strain M1390); AY548375 (AAS56918.1) (from N. meningitidis strain strain M6190); AY548373 (AAS56916.1) (from N. meningitidis strain 4243); and AY548372 (AAS56915.1) (from N. meningitidis strain BZ83).

it should be noted that the immature fHbp protein includes a leader sequence of about 19 residues. Furthermore, when provided an amino acid sequence the ordinarily skilled person can readily envision the sequences of nucleic acid that can encode for, and provide for expression of, a polypeptide having such an amino acid sequence.

In addition to the specific amino acid sequences and nucleic acid sequences provided herein, the disclosure also contemplates polypeptides and nucleic acids having sequences that are at least 80%, at least 85%, at least 90%, or at least 95% identical in sequence to the amino acid and nucleic acids. The terms "identical" or percent "identity," in the context of two or more polynucleotide sequences, or two or more amino acid sequences, refers to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same (e.g., at least 80%, at least 85%, at least 90%, or at least 95% identical over a specified region), when compared and aligned for maximum correspondence over a designated region, e.g., a  $V_E$  or a region at least about 40, 45, 50, 55, 60, 65 or more amino acids or nucleotides in length, and can be up to the full-length of the reference amino acid or nucleotide sequence (e.g., a full-length fHbp). The disclosure specifically contemplates both naturally-occurring polymorphisms and synthetically produced amino acid sequences and their encoding nucleic acids.

For sequence comparison, typically one sequence acts as a reference sequence (e.g., a naturally-occurring fHbp polypeptide sequence), to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer program, sequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program param-

Examples of algorithms that are suitable for determining percent sequence identity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al. (1990) J. Mol. Biol. 215: 403-410 and Altschul et al. (1977) Nucleic Acids Res. 25: 3389-3402, respectively. Software for per-

forming BLAST analyses is publicly available through the National Center for Biotechnology Information ("www" followed by ".ncbi.nlm.nih" followed by ".gov"). Further exemplary algorithms include ClustalW (Higgins D., et al. (1994) Nucleic Acids Res 22: 4673-4680), available at "www" fol-5 lowed by ".ebi.ac.uk/Tools/clustalw/index" followed by ".html".

In one embodiment, residue positions which are not identical differ by conservative amino acid substitutions. Conservative amino acid substitutions refer to the interchangeability 10 of residues having similar side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methion- 20

Sequence identity between two nucleic acids can also be described in terms of hybridization of two molecules to each other under stringent conditions. The hybridization conditions are selected following standard methods in the art (see, 25 for example, Sambrook, et al., Molecular Cloning: A Laboratory Manual, Second Edition, (1989) Cold Spring Harbor, N.Y.). An example of stringent hybridization conditions is hybridization at 50° C. or higher and 0.1×SSC (15 mM sodium chloride/1.5 mM sodium citrate). Another example of 30 stringent hybridization conditions is overnight incubation at 42° C. in a solution: 50% formamide, 5×SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5×Denhardt's solution, 10% dextran sulfate, and 20 mg/ml denatured, sheared salmon sperm DNA, followed by washing 35 the filters in 0.1×SSC at about 65° C. Stringent hybridization conditions are hybridization conditions that are at least as stringent as the above representative conditions, where conditions are considered to be at least as stringent if they are at gent as the above specific stringent conditions.

The chimeric fHbps of the present disclosure are described in more detail below in the context of modular architecture described above.

## Chimeric Factor H Binding Proteins

The chimeric fHbp of the present disclosure refers to nonnaturally occurring chimeric fHbp, including fHbps that may be described by a modular architecture as those set forth in FIG. 8, panels A and B and a modular architecture not shown in FIG. 8, panels A and B. The term "chimeric factor H 50 binding protein" or "chimeric fHbp" refers to a polypeptide comprising, from N-terminus to C-terminus, a group of five variable segments represented by  $V_A$ ,  $V_B$ ,  $V_C$ ,  $V_D$ , and  $V_E$ , in which the combination of  $\alpha/\beta$  progenitor segments and/or combination of alleles of which is not found in nature. One 55 example of the chimeric fHbps of the present disclosure can contain at least one or more of the variable segments derived from a different progenitor than at least one other variable segment in the chimeric fHbp. In other words, the chimeric fHbp contains at least one variable segment of an α progenitor 60 amino acid sequences and at least one variable segment of a β progenitor, such that the combination is not found in nature. In another example, the fHbp may also be of a modular architecture shown in FIG. 8, panel A (e.g. modular I or II in which all variable segments are  $\alpha$  or  $\beta$ , respectively), in which 65 each segment is of an allele such that the combination of the alleles is not found in nature.

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The variable segments may be flanked by invariant segments (I2, I3, I4, I5, I6, I7), which can act as linkers for the variable segments. Accordingly, the chimeric fHbps of the present disclosure can be described by the formula:

$$V_A$$
- $I_3$ - $V_B$ - $I_4$ - $V_C$ - $I_5$ - $V_D$ - $I_6$ - $V_E$ 

wherein each of the variable segments  $(V_A, V_B, V_C, V_D,$  and  $V_E$ ) can be derived from either an  $\alpha$  or a  $\beta$  progenitor amino acid sequence.

Chimeric fHbp can optionally include a leader sequence, e.g., to provide for expression of the chimeric fHbp on a cell surface of a bacterial host cell. Chimeric fHbp may also include about 1, 5, 10, or more residues as an N-terminal element (Nte) and/or C-terminal element (Cte). For example, the Nte may comprise 1, 2, or 6 glycine residues, or about 6 residues of a mixture of glycines and serines following the N-terminal most invariant segment  $(I_1)$ .

In addition, chimeric fHbps of the present disclosure can optionally include an N-terminal element, one or both of invariant segments that flank the N-terminal element, and/or most C-terminal invariant segment (I<sub>7</sub>). A chimeric fHbp comprising one or more of these optional features can be described by the following formula:

$$\begin{split} &\mathbf{I}_{2}\text{-}\mathbf{V}_{A}\text{-}\mathbf{I}_{3}\text{-}\mathbf{V}_{B}\text{-}\mathbf{I}_{4}\text{-}\mathbf{V}_{C}\text{-}\mathbf{I}_{5}\text{-}\mathbf{V}_{D}\text{-}\mathbf{I}_{6}\text{-}\mathbf{V}_{E};\\ &\text{Nte-}\mathbf{I}_{2}\text{-}\mathbf{V}_{A}\text{-}\mathbf{I}_{3}\text{-}\mathbf{V}_{B}\text{-}\mathbf{I}_{4}\text{-}\mathbf{V}_{C}\text{-}\mathbf{I}_{5}\text{-}\mathbf{V}_{D}\text{-}\mathbf{I}_{6}\text{-}\mathbf{V}_{E};\\ &\mathbf{I}_{1}\text{-}\text{Nte-}\mathbf{I}_{2}\text{-}\mathbf{V}_{A}\text{-}\mathbf{I}_{3}\text{-}\mathbf{V}_{B}\text{-}\mathbf{I}_{4}\text{-}\mathbf{V}_{C}\text{-}\mathbf{I}_{5}\text{-}\mathbf{V}_{D}\text{-}\mathbf{I}_{6}\text{-}\mathbf{V}_{E};\\ &\mathbf{V}_{A}\text{-}\mathbf{I}_{3}\text{-}\mathbf{V}_{B}\text{-}\mathbf{I}_{4}\text{-}\mathbf{V}_{C}\text{-}\mathbf{I}_{5}\text{-}\mathbf{V}_{D}\text{-}\mathbf{I}_{6}\text{-}\mathbf{V}_{E}\text{-}\mathbf{I}_{7};\\ &\mathbf{I}_{2}\text{-}\mathbf{V}_{A}\text{-}\mathbf{I}_{3}\text{-}\mathbf{V}_{B}\text{-}\mathbf{I}_{4}\text{-}\mathbf{V}_{C}\text{-}\mathbf{I}_{5}\text{-}\mathbf{V}_{D}\text{-}\mathbf{I}_{6}\text{-}\mathbf{V}_{E}\text{-}\mathbf{I}_{7};\\ &\mathbf{Nte-}\mathbf{I}_{2}\text{-}\mathbf{V}_{A}\text{-}\mathbf{I}_{3}\text{-}\mathbf{V}_{B}\text{-}\mathbf{I}_{4}\text{-}\mathbf{V}_{C}\text{-}\mathbf{I}_{5}\text{-}\mathbf{V}_{D}\text{-}\mathbf{I}_{6}\text{-}\mathbf{V}_{E}\text{-}\mathbf{I}_{7};\\ &\mathbf{Nte-}\mathbf{I}_{2}\text{-}\mathbf{V}_{A}\text{-}\mathbf{I}_{3}\text{-}\mathbf{V}_{B}\text{-}\mathbf{I}_{4}\text{-}\mathbf{V}_{C}\text{-}\mathbf{I}_{5}\text{-}\mathbf{V}_{D}\text{-}\mathbf{I}_{6}\text{-}\mathbf{V}_{E}\text{-}\mathbf{I}_{7};\\ \end{aligned}$$

 ${\rm I}_{1}\text{-}{\rm Nte}\text{-}{\rm I}_{2}\text{-}{\rm V}_{\mathcal{A}}\text{-}{\rm I}_{3}\text{-}{\rm V}_{\mathcal{B}}\text{-}{\rm I}_{4}\text{-}{\rm V}_{\mathcal{C}}\text{-}{\rm I}_{5}\text{-}{\rm V}_{\mathcal{D}}\text{-}{\rm I}_{6}\text{-}{\rm V}_{\mathcal{E}}\text{-}{\rm I}_{7}.$ 

in which the variable segments  $(\mathbf{V}_{\!A}, \mathbf{V}_{\!B}, \mathbf{V}_{\!C}, \mathbf{V}_{\!D}, \text{ and } \mathbf{V}_{\!E})$  can least about 80% as stringent, typically at least 90% as strin- 40 be derived from either an α or β progenitor amino acid sequence.

In addition to having various combinations of variable segments, each derived from either an  $\alpha$  or  $\beta$  progenitor amino acid sequence, chimeric fHbp of the present disclosure may also contain one or more variable segments that are heterologous with respect to  $\alpha$  and  $\beta$  progenitor amino acid sequences. A heterologous variable segment refers to a variable segment that comprises both a contiguous  $\alpha$  and a contiguous  $\beta$  progenitor amino acid sequences, with the point at which the amino acid sequence "switches" to the other progenitor sequence referred to herein as a "junction point". E.g. See  $V_A$  of peptide ID 55,  $V_B$  of peptide ID 24 and ID 25, and  $V_E$  of peptide ID 82. Particularly, one or more of the junction points (J<sub>1</sub>, J<sub>2</sub>, or J<sub>3</sub>) within a variable segment described above for peptide ID 55, 24, 25, and 82 may be used to construct a chimeric fHbp having a heterologous variable segment. See table in FIG. 8, panel C for the conserved residues in each variable segment that serve as junction points.

Variable segments of the non-naturally occurring chimeric fHbps of the present disclosure may have an amino acid sequence of at least 80%, at least 85%, at least 90%, at least 95% identical to a corresponding segment in a naturally occurring fHbp, and can be 100% identical in amino acid sequence.

Examples of chimeric fHbps include those containing one or more of the following variable segments. Each segment can be independently selected from any alleles in the corre-

sponding segment shown in FIGS. 2A-2E, Table 8. The variable segments are described in more detail below using certain alleles as examples.

 $V_A$  contains a contiguous amino acid sequence that is at least 85%, at least 90%, or at least 95% identical to Segment 5 ID A.α.1 or A.β.1 (SEQ ID NO:15 or NO:67) shown in FIG. 2A (Table 8), e.g., at least 85%, at least 90%, or at least 95% identical to one of the amino acid sequences shown in FIG. **2**A (Table 8) for Segment A.

 $V_B$  contains a contiguous amino acid sequence that is at 10 least 75%, at least 80%, at least 90%, or at least 95% identical to Segment ID B.α.1 or B.β.1 (SEQ ID NO:85 or NO:95) shown in FIG. 2B (Table 8), e.g., at least 75%, at least 80%, at least 90%, or at least 95% identical to one of the amino acid sequences shown in FIG. 2B (Table 8) for Segment B.

V<sub>C</sub> contains s a contiguous amino acid sequence that is at least 80%, at least 85%, at least 90%, or at least 95% identical to Segment ID C.a.1 or C.\u00e1.1 (SEQ ID NO:96 or NO:152) shown in FIG. 2C (Table 8), e.g., at least 80%, at least 85%, at least 90%, or at least 95% identical to one of the amino acid 20 sequences shown in FIG. 2C (Table 8) for Segment C.

 $V_D$  contains a contiguous amino acid sequence that is at least 80%, at least 85%, at least 90%, or at least 95% identical to Segment ID D.α.1 or D.β.1 (SEQ ID NO:174 or NO:192) at least 90%, or at least 95% identical to one of the amino acid sequences shown in FIG. **2**D (Table 8) for Segment D.

 $V_E$  contains a contiguous amino acid sequence that is at least 80%, at least 85%, at least 90%, or at least 95% identical to Segment ID E.α.1 or E.β.1 (SEQ ID NO:195 or NO:262) 30 shown in FIG. 2E (Table 8), e.g., at least 80%, at least 85%, at least 90%, or at least 95% identical to one of the amino acid sequences shown in FIG. 2E (Table 8) for Segment E.

The variable segments in the chimeric fHbp of the present disclosure may also be of the similar length as the correspond-35 ing segments listed in FIG. 2A-2E (Table 8). For example, a variable segment may be no more than about 50, 30, 20, 10, 5, or 1 amino acid residues less or more than one of the corresponding amino acid sequences shown in FIG. 2A-2E (Table

The chimeric fHbps of the present disclosure have a full length amino acid sequence that is not found in a naturally occurring fHbp. Most of the naturally occurring fHbps which contain only α or only β progenitor sequences or combinations thereof fall into one of the following modular groups: I, 45 II, III, IV, V, VI, VII, VIII, or IX (see FIG. 8). Non-naturally occurring fHbps of the present disclosure can also be of a modular group other than I, II, III, IV, V, VI, VII, VIII, or IX. The non-naturally occurring fHbps of the present disclosure can be described by new modular groups encompassing non- 50 naturally occurring amino acid sequences that are built based on the modular architecture described herein. Some examples of chimeric fHbps of the present disclosure are presented below.

Examples of Chimeric fHbps

The chimeric fHbps of the present disclosure encompass those that can be described in terms of one or more of, for example, the invariant and variable segments and their progenitor type, the presence or absence of an epitope(s) specifically bound by a mAb, or any combination of such features 60 that may be present in the chimeric fHbps.

Many combinations may be generated by switching  $\alpha$  or  $\beta$ segments in and out of various segments for a different type of modular group. Any alleles of  $\alpha$  or  $\beta$  segments can also be independently chosen from those shown FIG. 2A-2E (Table 8). Modular group may also be designed to maintain or include certain antibody epitopes. Certain antibodies are

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found to be bactericidal against a specific variant of N. meningitidis but not a different variant. If a chimeric fHbp of a modular group contains variable segments that present multiple epitopes, each specific for an antibody bactericidal against a different variant, the chimeric fHbp may be useful to elicit antibodies or immune response effective against a broad spectrum of N. meningitidis. For example, in a case where epitopes for JAR4, JAR3, and JAR32 antibodies are known and are desirable to be incorporated into an fHbp, a chimeric fHbp may be made to comprise  $V_A \alpha$ ,  $V_C \alpha$ , and  $V_D \beta$ , where epitopes of JAR4, JAR3, and JAR32 reside, respectively.

For example, a chimeric fHbp may have V<sub>D</sub> that is derived from  $\beta$  progenitor sequences while the rest of the variable segments remain as derivations from  $\alpha$  progenitor sequences. Such chimeric fHbp comprises variable segments represented as  $V_A \alpha$ ,  $V_B \alpha$ ,  $V_C \alpha$ ,  $V_D \beta$ , and  $V_E \alpha$ ; and its first and second junction points are at I<sub>5</sub> and I<sub>6</sub>, respectively. In a different example, an fHbp belonging to another modular group encompassing non-naturally occurring chimeric fHbp may contain  $V_A \alpha$ ,  $V_B \alpha$ ,  $V_C \alpha$ ,  $V_D \beta$ , and  $V_E \beta$ ; and its junction point resides at I<sub>5</sub>. An additional example of a modular group encompasses non-naturally occurring fHbp that comprises  $V_A \alpha, V_B \alpha, V_C \alpha, V_D \alpha$ , and  $V_E \beta$  as the invariable segments.

Accordingly, examples of non-naturally occurring chishown in FIG. 2D (Table 8), e.g., at least 80%, at least 85%, 25 meric fHbps including those described above may be of modular groups represented by the following formula but not limited to:

$$\begin{split} & V_{\mathcal{A}} \alpha \text{-} I_{3} \text{-} V_{\mathcal{B}} \alpha \text{-} I_{4} \text{-} V_{\mathcal{C}} \alpha \text{-} I_{5} \text{-} V_{\mathcal{D}} \beta \text{-} I_{6} \text{-} V_{\mathcal{E}} \alpha; \\ & V_{\mathcal{A}} \alpha \text{-} I_{3} \text{-} V_{\mathcal{B}} \alpha \text{-} I_{4} \text{-} V_{\mathcal{C}} \alpha \text{-} I_{5} \text{-} V_{\mathcal{D}} \beta \text{-} I_{6} \text{-} V_{\mathcal{E}} \beta; \\ & V_{\mathcal{A}} \alpha \text{-} I_{3} \text{-} V_{\mathcal{B}} \alpha \text{-} I_{4} \text{-} V_{\mathcal{C}} \alpha \text{-} I_{5} \text{-} V_{\mathcal{D}} \alpha \text{-} I_{6} \text{-} V_{\mathcal{E}} \beta; \\ & V_{\mathcal{A}} \alpha \text{-} I_{3} \text{-} V_{\mathcal{B}} \alpha \text{-} I_{4} \text{-} V_{\mathcal{C}} \beta \text{-} I_{5} \text{-} V_{\mathcal{D}} \text{-} I_{6} \text{-} V_{\mathcal{E}} \alpha; \\ & V_{\mathcal{A}} \alpha \text{-} I_{3} \text{-} V_{\mathcal{B}} \alpha \text{-} I_{4} \text{-} V_{\mathcal{C}} \beta \text{-} I_{5} \text{-} V_{\mathcal{D}} \text{-} I_{6} \text{-} V_{\mathcal{E}} \beta; \\ & V_{\mathcal{A}} \beta \text{-} I_{3} \text{-} V_{\mathcal{B}} \alpha \text{-} I_{4} \text{-} V_{\mathcal{C}} \alpha \text{-} I_{5} \text{-} V_{\mathcal{D}} \alpha \text{-} I_{6} \text{-} V_{\mathcal{E}} \beta; \\ & V_{\mathcal{A}} \beta \text{-} I_{3} \text{-} V_{\mathcal{B}} \alpha \text{-} I_{4} \text{-} V_{\mathcal{C}} \alpha \text{-} I_{5} \text{-} V_{\mathcal{D}} \beta \text{-} I_{6} \text{-} V_{\mathcal{E}} \alpha; \\ & V_{\mathcal{A}} \beta \text{-} I_{3} \text{-} V_{\mathcal{B}} \alpha \text{-} I_{4} \text{-} V_{\mathcal{C}} \beta \text{-} I_{5} \text{-} V_{\mathcal{D}} \alpha \text{-} I_{6} \text{-} V_{\mathcal{E}} \alpha; \\ & V_{\mathcal{A}} \beta \text{-} I_{3} \text{-} V_{\mathcal{B}} \beta \text{-} I_{4} \text{-} V_{\mathcal{C}} \beta \text{-} I_{5} \text{-} V_{\mathcal{D}} \alpha \text{-} I_{6} \text{-} V_{\mathcal{E}} \alpha; \\ & V_{\mathcal{A}} \beta \text{-} I_{3} \text{-} V_{\mathcal{B}} \beta \text{-} I_{4} \text{-} V_{\mathcal{C}} \beta \text{-} I_{5} \text{-} V_{\mathcal{D}} \alpha \text{-} I_{6} \text{-} V_{\mathcal{E}} \alpha; \\ & V_{\mathcal{A}} \beta \text{-} I_{3} \text{-} V_{\mathcal{B}} \beta \text{-} I_{4} \text{-} V_{\mathcal{C}} \beta \text{-} I_{5} \text{-} V_{\mathcal{D}} \alpha \text{-} I_{6} \text{-} V_{\mathcal{E}} \alpha; \\ & V_{\mathcal{A}} \beta \text{-} I_{3} \text{-} V_{\mathcal{B}} \beta \text{-} I_{4} \text{-} V_{\mathcal{C}} \beta \text{-} I_{5} \text{-} V_{\mathcal{D}} \beta \text{-} I_{6} \text{-} V_{\mathcal{E}} \alpha; \\ & V_{\mathcal{A}} \beta \text{-} I_{3} \text{-} V_{\mathcal{B}} \beta \text{-} I_{4} \text{-} V_{\mathcal{C}} \beta \text{-} I_{5} \text{-} V_{\mathcal{D}} \beta \text{-} I_{6} \text{-} V_{\mathcal{E}} \alpha; \\ & V_{\mathcal{A}} \beta \text{-} I_{3} \text{-} V_{\mathcal{B}} \beta \text{-} I_{4} \text{-} V_{\mathcal{C}} \beta \text{-} I_{5} \text{-} V_{\mathcal{D}} \beta \text{-} I_{6} \text{-} V_{\mathcal{E}} \alpha; \\ & V_{\mathcal{A}} \beta \text{-} I_{3} \text{-} V_{\mathcal{B}} \beta \text{-} I_{4} \text{-} V_{\mathcal{C}} \beta \text{-} I_{5} \text{-} V_{\mathcal{D}} \beta \text{-} I_{6} \text{-} V_{\mathcal{E}} \alpha; \\ & V_{\mathcal{A}} \beta \text{-} I_{3} \text{-} V_{\mathcal{B}} \beta \text{-} I_{4} \text{-} V_{\mathcal{C}} \beta \text{-} I_{5} \text{-} V_{\mathcal{D}} \beta \text{-} I_{6} \text{-} V_{\mathcal{E}} \alpha; \\ & V_{\mathcal{A}} \beta \text{-} I_{3} \text{-} V_{\mathcal{B}} \beta \text{-} I_{4} \text{-} V_{\mathcal{C$$

In any group shown above in which a variable segment  $(V_A)$  $V_B, V_C, V_D, \text{ or } V_E)$  is not followed by an  $\alpha$  or a  $\beta$  (e.g.  $V_C, V_D$ , and  $V_E$  in the fifth modular group from the top) represents a variable segment that can be derived from either an  $\alpha$  or a  $\beta$ progenitor amino acid sequence. The formula may also include a variable N-terminal element, its flanking invariable sequences (I<sub>1</sub> and I<sub>2</sub>), or the most C-terminal invariable segment  $(I_7)$ , omitted in the formula above. As noted above for fHbp of the present disclosure, each variable segment of the fHbps described by the formulas above can be of any allele.

In another example, the chimeric fHbp of the present disclosure also encompass those that contain all  $\alpha$  or all  $\beta$  segments, in which each of the segments is of an allele such that the combination of all  $\alpha$  or all  $\beta$  allelic segments is not found in nature.

Chimeric fHbp Comprising Heterologous Variable Segment

In certain cases, non-naturally occurring fHbp of the present disclosure may comprise one or more heterologous variable segments. These heterologous variable segmentcontaining fHbps may also be described by modular architectures other than those set forth in FIG. 8, panel A. Thus, for

example, non-naturally occurring fHbp of the present disclosure include those having: a heterologous  $V_A(\beta \rightarrow \alpha)$ , but do not have  $\alpha$  progenitor sequence for all of  $V_B$ ,  $V_C$ ,  $V_D$ , and  $V_E$ ; a heterologous  $V_B(\alpha \rightarrow \beta)$ , but do not have a  $V_A$  of  $\alpha$  progenitor sequence together with  $V_C$ ,  $V_D$ , and  $V_E$  of  $\beta$  progenitor 5 sequence; and a heterologous  $V_E(\alpha \rightarrow \beta)$ , but do not have  $\beta$ progenitor sequence for all of  $V_A$ ,  $V_B$ , and  $V_C$  together with  $\alpha$ progenitor sequence for  $V_D$ . Exemplary non-naturally occurring fHbps are presented by several examples below, each containing one heterologous variable segment. However, 10 non-naturally occurring fHbps are not limited to the examples, e.g. an fHbp may comprise more than one heterologous variable segment.

For example, a heterologous variable segment-containing fHbp may be one comprising a heterologous  $V_A$ , such as  $V_A(\beta \rightarrow \alpha)$  as exemplified by peptide ID 55, in which not all of  $V_B$ ,  $V_C$ ,  $V_D$ , and  $V_E$  are of the  $\alpha$  progenitor amino acid sequences. A non-naturally occurring fHbp may also comprise  $V_A(\alpha \rightarrow \beta)$ , and in which  $V_B$ ,  $V_C$ ,  $V_D$ , and  $V_E$  are independently selected from  $\alpha$  and  $\beta$ . Another exemplary nonnaturally occurring fHbp may comprise  $V_B(\alpha \rightarrow \beta)$  as exemplified by peptide ID 24, in which  $V_A$  is of a  $\alpha$  progenitor and not all of  $V_C$ ,  $V_D$ , and  $V_E$  are of the  $\beta$  progenitor amino acid sequences. A non-naturally occurring fHbp may comprise  $V_B(\alpha \rightarrow \beta)$ , in which  $V_A$  is of a  $\beta$  progenitor. A non- 25 naturally occurring fHbp may comprise  $V_B(\beta \rightarrow \alpha)$  in which  $V_A$ ,  $V_C$ ,  $V_D$ , and  $V_E$  are independently selected from  $\alpha$  and  $\beta$ . Additionally, a non-naturally occurring fHbp may comprise a heterologous  $V_E$ , such as  $V_E(\alpha \rightarrow \beta)$  seen in peptide ID 82, together with  $V_D \alpha$ , in which not all of  $V_A$ ,  $V_B$ ,  $V_C$  are of the  $\beta$  30 progenitor origin. A non-naturally occurring fHbp may comprise  $V_E(\alpha \rightarrow \beta)$  together with  $V_D\beta$ , in which  $V_A$ ,  $V_B$ , and  $V_C$ are independently selected from  $\alpha$  and  $\beta$ . Another example of a non-naturally occurring fHbp that contains a heterologous variable segment is one that comprises  $V_E(\beta \rightarrow \alpha)$ , in which 35  $V_A, V_B, V_C$ , and  $V_D$  are independently selected from  $\alpha$  and  $\beta$ . Chimeric fHbp Having Modified Epitope(s)

The chimeric fHbp of the present disclosure may comprise one or more variable segments in which one or more residues may be mutated relative to the amino acid sequence found in 40 the naturally occurring fHbp so as to introduce an epitope of interest. These epitopes are referred to herein "heterologous epitopes" since they are heterologous to the variable segment in which they reside. Site-directed mutagenesis may be used antibody of interest into a variable segment that naturally does not contain such epitope. As such, mutations to insert, delete, or substitute one or more amino acid residues may be used to create chimeric fHbp of the present disclosure. Various mutations may be also screened for an fHbp that is effec- 50 tive as vaccines or may be immunogenic to elicit bactericidal antibodies.

For example, an fHbp may comprise an epitope that binds to an antibody JAR13, normally found in  $V_E$  of a  $\beta$  progenitor sequence and not in an  $\alpha$  progenitor sequence. To generate a 55 fHbp that includes  $\mathbf{V}_{\!\scriptscriptstyle E}\!\alpha$  while at the same time contains the JAR13 epitope, the epitope may be introduced into a  $V_F\alpha$ sequence. Selective mutation allows the amino acid sequence of  $V_E$  to be derived from the  $\alpha$  progenitor type except for a specific site for the JAR13 epitope. As such, regardless of the 60 amino acid sequence used to derive the sequences of the variable segments of the chimeric fHbp, epitopes may be introduced into the corresponding variable segment to include or maintain regions of desired antigenicity.

Chimeric fHbps also encompass chimeric fHbp that con- 65 tain two or more epitopes that elicit antibodies that, when both are bound to their respective epitopes, exhibit enhanced

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bactericidal activity against N. meningitidis than when either one is bound alone. For example, the combination of JAR 4 and JAR 5 mAbs exhibit higher level of bactericidal activity against N. meningitidis than when each is used alone in a human-complement-mediated assay. Chimeric fHbp can be designed so as to ensure that such epitopes are maintained or to introduce such combination of epitopes. Chimeric fHbps can also be designed to include an epitope(s) that elicits antibodies that, when bound to fHbp, inhibit fH binding. For example, when the epitopes bound by the monoclonal antibodies JAR3, JAR 5, JAR11, or JAR32/35 are bound by antibody, binding of fHbp to fH is inhibited. Thus, the presence of such fH-binding epitopes in the chimeric fHbp polypeptides can provide for production of antibodies that can facilitate protection through this pathway.

The antibodies that bind epitopes that may be of interest to introduce or maintain in the segments of a chimeric fHbp are disclosed in WO 09/114,485, the disclosure of which is incorporated in its entirety by reference. Certain epitopes of interest with a corresponding JAR mAbs are presented in the table 1 below and pointed out in FIG. 9, panel C.

TABLE 1

/T		
(Immunogen) Antibody	Reactive Residue(s)	fHbp variant bound
mAb 502	R204	1
JAR 3	G121 and K122	1
JAR 4	DHK (starting at 25);	v.1, v.2 (high reactivity)
	YGN (starting at 57)	and v.3 (lower reactivity)
JAR 5	G121 and K122	v.1
JAR 10	K180 and E192	v.1 (subset), v.2 (subset) and v. 3 (subset)
JAR 11	A174	v.2 (subset) and v.3 (subset)
JAR 13	S216	v. 2 (subset) and v.3 (all)
JAR 32	K174	v. 3 and v.2 (subset)
JAR 33	E180 and R192	v. 3 and v.2 (subset)
JAR 35	K174	v. 3 and v.2 (subset)

Other Features of Interest

Chimeric polypeptides described herein can include addito introduce one or more heterologous epitopes for a specific 45 tional heterologous amino acid sequences, e.g., to provide an N-terminal methionine or derivative thereof (e.g., pyroglutamate) as a result of expression in a bacterial host cell (e.g., E. coli) and/or to provide a chimeric polypeptide having a fusion partner at its N-terminus or C-terminus. Fusion partners of interest include, for example, glutathione-S-transferase (GST), maltose binding protein (MBP),  $\operatorname{His}_6$ -tag, and the like, as well as leader peptides from other proteins, particularly lipoproteins. Fusion partners can provide for additional features, such as in facilitating isolation and purification of the chimeric polypeptide.

Native fHbp usually contains an N-terminal cysteine to which a lipid moiety can be covalently attached. This cysteine residue is usually lipidated in the naturally-occurring protein, and can be lipidated in the chimeric fHbps disclosed herein. Thus, in the amino acid sequences described herein (including those presented in any Sequence Listing), reference to "cysteine" or "C" at this position specifically includes reference to both an unmodified cysteine as well as to a cysteine that is lipidated (e.g., due to post-translational modification). Thus, the chimeric fHbp can be lipidated or non-lipidated. Methods for production of lipidated proteins in vitro, (see, e.g., Andersson et al., 2001 J. Immunological Methods 255

(1-2):135-48) or in vivo are known in the art. For example, lipidated fHbp previously has been purified from the membrane fraction of *E. coli* protein by detergent extraction (Fletcher et al., 2004 Infection and Immunity 72(4):2088-100), which method may be adapted for the production of blipidated chimeric fHbp. Lipidated proteins may be of interest as such can be more immunogenic than soluble protein (see, e.g., Fletcher et al., 2004 Infection and Immunity 72(4):2088-100)

Nucleic Acid Encoding Chimeric fHBP

The chimeric fHbp can be generated using recombinant techniques to manipulate nucleic acids of different fHbps known in the art to provide constructs encoding a chimeric fHbp of interest. As noted above, nucleic acids encoding a variety of different v.1, v.2, and v.3 fHbps of *N. meningitidis* are available in the art, and their nucleotide sequences are known.

Amino acid and nucleic acid sequences of the naturally occurring fHbps are provided in FIG. **2**A-**2**E (Table 5) and 20 available in the art. It will be appreciated that the nucleotide sequences encoding the chimeric fHbps can be modified so as to optimize the codon usage to facilitate expression in a host cell of interest (e.g., *E. coli, N. meningitidis*, human (as in the case of a DNA-based vaccine), and the like). Methods for 25 production of codon optimized sequences are known in the art.

### Methods of Screening

The present disclosure also features methods of screening for an immunogenic composition comprising a chimeric 30 fHbp, vaccine against bacterial pathogens, antibodies and nucleic acid encoding the same. The methods may involve evaluating the ability of a specific chimeric fHbp to elicit bactericidal antibodies and/or to provide a broad spectrum immunity against bacterial pathogens. The methods can 35 involve assessing the ability of antibodies elicited by immunization with a chimeric fHbp of the present disclosure to inhibit fH binding to fHbp of N. meningitidis of diverse variant groups and/or to elicit bactericidal activity. The antibody may be elicited in a host animal immunized with an immu- 40 nogenic composition comprising a chimeric fHbp of the present disclosure or screened in a phage display library for its specific affinity to a candidate chimeric fHbp. Among other aspects, the methods find particular use in identifying and/or evaluating antibody having bactericidal and/or anti- 45 neoplastic activity.

In one embodiment, a method of evaluating binding of an antibody to a bacterial cell is provided. The method comprises: (a) immunizing a host animal with a composition comprising a chimeric fHbp of the present disclosure, (b) 50 isolating antibodies from the host animal that have binding affinity to the chimeric fHbp, (c) contacting a bacterial cell with the isolated antibodies; and (d) assessing binding of the antibody to the bacterial cell. Additional steps may include assessing the competitive binding of the antibody to fHbp 55 with human factor H; assessing the bactericidal activity against a bacterial pathogen when incubated in vitro with complement; or assessing the ability of the antibody administered to an animal to confer passive protection against infection. In some embodiments, the antibody is in an antibody 60 population, and the method further comprises: (c) isolating one or more antibodies of the antibody population that bind the bacterial cell. A featured aspect is isolated antibody that is bactericidal against the bacterial cell, which may include, for example, complement-mediated bactericidal activity and/or 65 opsonophagocytic activity capable of decreasing the viability of the bacteria in human blood. The subject method may also

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include assessing the susceptibility of a host animal administered with a vaccine comprising a chimeric fHbp to a bacterial pathogen.

Bacterial pathogen of particular interest are *N. meningitidis* of any or all fHbp variant groups, of diverse capsular groups, such as *N. meningitidis* Serogroup B, *N. meningitidis* Serogroup C, *N. meningitidis* Serogroup X, *N. meningitidis* Serogroup Y, *N. meningitidis* Serogroup W-135, and the like. Methods of Production

Chimeric fHbps can be produced by any suitable method, including recombinant and non-recombinant methods (e.g., chemical synthesis). Where the chimeric fHbp is produced using recombinant techniques, the methods can involve any suitable construct and any suitable host cell, which can be a prokaryotic or eukaryotic cell, usually a bacterial or yeast host cell, more usually a bacterial cell. Methods for introduction of genetic material into host cells include, for example, transformation, electroporation, conjugation, calcium phosphate methods and the like. The method for transfer can be selected so as to provide for stable expression of the introduced chimeric fHbp-encoding nucleic acid. The chimeric fHbp-encoding nucleic acid can be provided as an inheritable episomal element (e.g., plasmid) or can be genomically integrated.

Suitable vectors for transferring chimeric fHbp-encoding nucleic acid can vary in composition. Integrative vectors can be conditionally replicative or suicide plasmids, bacteriophages, and the like. The constructs can include various elements, including for example, promoters, selectable genetic markers (e.g., genes conferring resistance to antibiotics (for instance kanamycin, erythromycin, chloramphenicol, or gentamycin)), origin of replication (to promote replication in a host cell, e.g., a bacterial host cell), and the like. The choice of vector will depend upon a variety of factors such as the type of cell in which propagation is desired and the purpose of propagation. Certain vectors are useful for amplifying and making large amounts of the desired DNA sequence. Other vectors are suitable for expression in cells in culture. Still other vectors are suitable for transfer and expression in cells in a whole animal. The choice of appropriate vector is well within the skill of the art. Many such vectors are available commercially.

The vector can be an expression vector based on episomal plasmids containing selectable drug resistance markers and elements that provide for autonomous replication in different host cells (e.g., in both *E. coli* and *N. meningitidis*). One example of such a "shuttle vector" is the plasmid pFP10 (Pagotto et al. Gene 2000 244:13-19).

Constructs can be prepared by, for example, inserting a polynucleotide of interest into a construct backbone, typically by means of DNA ligase attachment to a cleaved restriction enzyme site in the vector. Alternatively, the desired nucleotide sequence can be inserted by homologous recombination or site-specific recombination. Typically homologous recombination is accomplished by attaching regions of homology to the vector on the flanks of the desired nucleotide sequence, while site-specific recombination can be accomplished through use of sequences that facilitate site-specific recombination (e.g., cre-lox, att sites, etc.). Nucleic acid containing such sequences can be added by, for example, ligation of oligonucleotides, or by polymerase chain reaction using primers comprising both the region of homology and a portion of the desired nucleotide sequence.

Vectors can provide for extrachromosomal maintenance in a host cell or can provide for integration into the host cell genome. Vectors are amply described in numerous publications well known to those in the art, including, e.g., Short

Protocols in Molecular Biology, (1999) F. Ausubel, et al., eds., Wiley & Sons. Vectors may provide for expression of the nucleic acids encoding a chimeric fHBP, may provide for propagating the subject nucleic acids, or both.

Examples of vectors that may be used include but are not 5 limited to those derived from recombinant bacteriophage DNA, plasmid DNA or cosmid DNA. For example, plasmid vectors such as pBR322, pUC 19/18, pUC 118, 119 and the M13 mp series of vectors may be used. pET21 is also an expression vector that may be used. Bacteriophage vectors 10 may include λgt10, λgt11, λgt18-23, λZAP/R and the EMBL series of bacteriophage vectors. Further vectors that may be utilized include, but are not limited to, pJB8, pCV 103, pCV 107, pCV 108, pTM, pMCS, pNNL, pHSG274, COS202, COS203, pWE15, pWE16 and the charomid 9 series of vectors.

For expression of a chimeric fHbp of interest, an expression cassette may be employed. Thus, the present disclosure provides a recombinant expression vector comprising a subject nucleic acid. The expression vector provides transcrip- 20 tional and translational regulatory sequences, and may provide for inducible or constitutive expression, where the coding region is operably linked under the transcriptional control of the transcriptional initiation region, and a transcriptional and translational termination region. These control 25 regions may be native to an fHbp from which the chimeric fHbp is derived, or may be derived from exogenous sources. In general, the transcriptional and translational regulatory sequences may include, but are not limited to, promoter sequences, ribosomal binding sites, transcriptional start and 30 stop sequences, translational start and stop sequences, and enhancer or activator sequences. Promoters can be either constitutive or inducible, and can be a strong constitutive promoter (e.g., T7, and the like).

Expression vectors generally have convenient restriction 35 sites located near the promoter sequence to provide for the insertion of nucleic acid sequences encoding proteins of interest. A selectable marker operative in the expression host may be present to facilitate selection of cells containing the vector. In addition, the expression construct may include 40 additional elements. For example, the expression vector may have one or two replication systems, thus allowing it to be maintained in organisms, for example in mammalian or insect cells for expression and in a prokaryotic host for cloning and amplification. In addition the expression construct may contain a selectable marker gene to allow the selection of transformed host cells. Selection genes are well known in the art and will vary with the host cell used.

It should be noted that chimeric fHbp of the present disclosure may comprise additional elements, such as a detectable label, e.g., a radioactive label, a fluorescent label, a biotin label, an immunologically detectable label (e.g., an HA tag, a poly-Histidine tag) and the like. Additional elements of chimeric fHbp can be provided to facilitate isolation (e.g., biotin tag, immunologically detectable tag) through various methods (e.g., affinity capture, etc.). Chimeric fHbp can optionally be immobilized on a support through covalent or non-covalent attachment.

Isolation and purification of chimeric fHbp can be accomplished according to methods known in the art. For example, 60 chimeric fHbp can be isolated from a lysate of cells genetically modified to express a chimeric fHbp, or from a synthetic reaction mix, by immunoaffinity purification, which generally involves contacting the sample with an anti-chimeric fHbp antibody (e.g., an anti-chimeric fHBP mAb, such as a 65 JAR 5 mAb or other appropriate JAR mAb described herein), washing to remove non-specifically bound material, and elut-

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ing specifically bound chimeric fHbp. Isolated chimeric fHbp can be further purified by dialysis and other methods normally employed in protein purification methods. In one embodiment, the chimeric fHbp can be isolated using metal chelate chromatography methods.

Host Cells

Any of a number of suitable host cells can be used in the production of chimeric fHbp. In general, the chimeric fHbp described herein may be expressed in prokaryotes or eukaryotes, usually bacteria, more usually E. coli or Neisseria (e.g., N. meningitidis) in accordance with conventional techniques. Thus, the present disclosure further provides a genetically modified host cell, which contains a nucleic acid encoding a chimeric fHBP. Host cells for production (including large scale production) of a chimeric fHBP can be selected from any of a variety of available host cells. Exemplary host cells for expression include those of a prokaryotic or eukaryotic unicellular organism, such as bacteria (e.g., Escherichia coli strains), yeast (e.g., S. cerevisiae, Pichia spp., and the like), and may include host cells originally derived from a higher organism such as insects, vertebrates, particularly mammals, (e.g. CHO, HEK, and the like). Generally bacterial host cells and yeast are of particular interest for chimeric fHBP production.

Chimeric fHbps can be prepared in substantially pure or substantially isolated form (i.e., substantially free from other *Neisserial* or host cell polypeptides) or substantially isolated form. In certain embodiments, the chimeric fHbp is present in a composition that is enriched for the polypeptide relative to other components that may be present (e.g., other polypeptides or other host cell components). Purified chimeric fHbp can be provided such that the polypeptide is present in a composition that is substantially free of other expressed polypeptides, e.g., less than 90%, usually less than 60% and more usually less than 50% of the composition is made up of other expressed polypeptides.

Host Cells for Vesicle Production

Where a chimeric fHbp is to be provided in a membrane vesicle (as discussed in more detail below), a *Neisserial* host cell is genetically modified to express a chimeric fHbp. Any of a variety of *Neisseria* spp. strains can be modified to produce a chimeric fHbp, and, optionally, which produce or can be modified to produce other antigens of interest, such as PorA, can be used in the methods disclosed herein.

Methods and vectors to provide for genetic modification of *Neisserial* strains and expression of a desired polypeptide are known in the art. Examples of vectors and methods are provided in WO 02/09746 and O'Dwyer et al. Infect Immun 2004; 72:6511-80. Strong promoters, particularly constitutive strong promoters are of particular interest. Exemplary promoters include the promoters of porA, porB, lbpB, tbpB, p110, hpuAB, lgtF, opa, p110, 1st, hpuAB. and rmp.

Pathogenic *Neisseria* spp. or strains derived from pathogenic *Neisseria* spp., particularly strains pathogenic for humans or derived from strains pathogenic or commensal for humans, are of particular interest for use in membrane vesicle production. Examples of *Neisserial* spp. include *N. meningitidis*, *N. flavescens*, *N. gonorrhoeae*, *N. lactamica*, *N. polysaccharea*, *N. cinerea*, *N. mucosa*, *N. subflava*, *N. sicca*, *N. elongata*, and the like.

N. meningitidis strains are of particular interest for genetic modification to express a chimeric fHbp and for use in vesicle production. The strain used for vesicle production can be selected according to a number of different characteristics that may be desired. For example, the strain may be selected according to: a desired PorA type (a "serosubtype", as described above), capsular group, serotype, and the like;

decreased capsular polysaccharide production; and the like. For example, the production strain can produce any desired PorA polypeptide, and may express one or more PorA polypeptides (either naturally or due to genetic engineering). Examples of strains include those that produce a PorA 5 polypeptide which confers a serosubtype of P1.7,16; P1.19, 15; P1.7,1; P1.5,2; P1.22a,14; P1.14; P1.5,10; P1.7,4; P1.12, 13; as well as variants of such PorA polypeptides which may or may not retain reactivity with conventional serologic reagents used in serosubtyping. Also of interest are PorA polypeptides characterized according to PorA variable region (VR) typing (see, e.g., Russell et al. Emerging Infect Dis 2004 10:674-678; Sacchi CT, et al, Clin Diagn Lab Immunol 1998; 5:845-55; Sacchi et al, J. Infect Dis 2000; 182:1169-1176). A substantial number of distinct VR types have been identified, which can be classified into VR1 and VR2 family "prototypes". A web-accessible database describing this nomenclature and its relationship to previous typing schemes is found at neisseria.org/nm/typing/pora. Alignments of exemplary PorA VR1 and VR2 types are provided in Russell et al. 20 Emerging Infect Dis 2004 10:674-678.

Alternatively or in addition, the production strain can be a capsule deficient strain. Capsule deficient strains can provide vesicle-based vaccines that provide for a reduced risk of eliciting a significant autoantibody response in a subject to 25 whom the vaccine is administered (e.g., due to production of antibodies that cross-react with sialic acid on host cell surfaces). "Capsule deficient" or "deficient in capsular polysaccharide" as used herein refers to a level of capsular polysaccharide on the bacterial surface that is lower than that of a 30 naturally-occurring strain or, where the strain is genetically modified, is lower than that of a parental strain from which the capsule deficient strain is derived. A capsule deficient strain includes strains that are decreased in surface capsular polysaccharide production by at least 10%, 20%, 25%, 30%, 35 40%, 50%, 60%, 75%, 80%, 85%, 90% or more, and includes strains in which capsular polysaccharide is not detectable on the bacterial surface (e.g., by whole cell ELISA using an anti-capsular polysaccharide antibody).

Capsule deficient strains include those that are capsule 40 deficient due to a naturally-occurring or recombinantly-generated genetic modification. Naturally-occurring capsule deficient strains (see, e.g., Dolan-Livengood et al. J. Infect. Dis. (2003) 187(10):1616-28), as well as methods of identifying and/or generating capsule-deficient strains (see, e.g., 45 Fisseha et al. (2005) Infect. Immun. 73(7):4070-4080; Stephens et al. (1991) Infect Immun 59(11):4097-102; Frosch et al. (1990) Mol. Microbiol. 1990 4(7):1215-1218) are known in the art.

Modification of a *Neisserial* host cell to provide for 50 decreased production of capsular polysaccharide may include modification of one or more genes involved in capsule synthesis, where the modification provides for, for example, decreased levels of capsular polysaccharide relative to a parent cell prior to modification. Such genetic modifications can 55 include changes in nucleotide and/or amino acid sequences in one or more capsule biosynthesis genes rendering the strain capsule deficient (e.g., due to one or more insertions, deletions, substitutions, and the like in one or more capsule biosynthesis genes). Capsule deficient strains can lack or be 60 non-functional for one or more capsule genes.

Of particular interest are strains that are deficient in sialic acid biosynthesis. Such strains can provide for production of vesicles that have reduced risk of eliciting anti-sialic acid antibodies that cross-react with human sialic acid antigens, 65 and can further provide for improved manufacturing safety. Strains having a defect in sialic acid biosynthesis (due to

either a naturally occurring modification or an engineered modification) can be defective in any of a number of different genes in the sialic acid biosynthetic pathway. Of particular interest are strains that are defective in a gene product encoded by the N-acetylglucosamine-6-phosphate 2-epimerase gene (known as synX AAF40537.1 or siaA AAA20475), with strains having this gene inactivated being of especial interest. For example, in one embodiment, a capsule deficient strain is generated by disrupting production of a functional synX gene product (see, e.g., Swartley et al. (1994) J. Bacteriol. 176(5):1530-4).

Capsule-deficient strains can also be generated from naturally-occurring strains using non-recombinant techniques, e.g., by use of bactericidal anti-capsular antibodies to select for strains that reduced in capsular polysaccharide.

Where the disclosure involves use of two or more strains (e.g., to produce antigenic compositions containing a chimeric fHbp-presenting vesicles from different strains), the strains can be selected so as to differ in one or more strain characteristics, e.g., to provide for vesicles that differ in the chimeric fHbp used, PorA, and the like.

Preparation of Vesicles

The antigenic compositions contemplated by the present disclosure generally include vesicles prepared from *Neisserial* cells that express a chimeric fHbp. As referred to herein "vesicles" is meant to encompass outer membrane vesicles as well as microvesicles (which are also referred to as blebs).

The antigenic composition can contain outer membrane vesicles (OMV) prepared from the outer membrane of a cultured strain of Neisseria meningitidis spp. genetically modified to express a chimeric fHbp. OMVs may be obtained from Neisseria meningitidis grown in broth or solid medium culture, preferably by separating the bacterial cells from the culture medium (e.g. by filtration or by a low-speed centrifugation that pellets the cells, or the like), lysing the cells (e.g. by addition of detergent, osmotic shock, sonication, cavitation, homogenization, or the like) and separating an outer membrane fraction from cytoplasmic molecules (e.g. by filtration; or by differential precipitation or aggregation of outer membranes and/or outer membrane vesicles, or by affinity separation methods using ligands that specifically recognize outer membrane molecules; or by a high-speed centrifugation that pellets outer membranes and/or outer membrane vesicles, or the like); outer membrane fractions may be used to produce OMVs.

The antigenic composition can contain microvesicles (MV) (or "blebs") containing chimeric fHbp, where the MV or blebs are released during culture of a Neisseria meningitidis strain genetically modified to express a chimeric fHbp. For example, MVs may be obtained by culturing a strain of Neisseria meningitidis in broth culture medium, separating whole cells from the broth culture medium (e.g. by filtration, or by a low-speed centrifugation that pellets only the cells and not the smaller blebs, or the like), and then collecting the MVs that are present in the cell-free culture medium (e.g. by filtration, differential precipitation or aggregation of MVs, or by a high-speed centrifugation that pellets the blebs, or the like). Strains for use in production of MVs can generally be selected on the basis of the amount of blebs produced in culture (e.g., bacteria can be cultured in a reasonable number to provide for production of blebs suitable for isolation and administration in the methods described herein). An example of a strain that produces high levels of blebs is described in PCT Publication No. WO 01/34642. In addition to bleb production, strains for use in MV production may also be selected on the basis of NspA production, where strains that produce higher levels of NspA may be of particular interest (for examples of N. men-

ingitidis strains having different NspA production levels, see, e.g., Moe et al. (1999 Infect. Immun. 67: 5664). Other strains of interest for use in production of blebs include strains having an inactivated GN33 gene, which encodes a lipoprotein required for cell separation, membrane architecture and virulence (see, e.g., Adu-Bobie et al. Infect Immun 2004; 72:1914-1919).

The antigenic compositions of the present disclosure can contain vesicles from one strain, or from 2, 3, 4, 5 or more strains, which strains may be homologous or heterologous, 10 usually heterologous, to one another. For example, the strains may be homologous or heterologous with respect to PorA. The vesicles can also be prepared from strains that express more than one chimeric fHbp (e.g., 1, 2, 3, or more chimeric fHbp) which may be composed of fHbp amino acid 15 sequences from different variants (v.1, v.2, or v.3) or subvariants (e.g., a subvariant of v.1, v.2, or v.3).

The antigenic compositions can contain a mixture of OMVs and MVs presenting the same or different chimeric fHbps, where the chimeric fHbps may optionally present 20 epitopes from different combinations of fHbp variants and/or subvariants and where the OMVs and/or MVs may be from the same or different strains. Vesicles from different strains can be administered as a mixture, or can be administered serially. The antigentic composition may contain vesicles 25 containing one or more different chimeric fHbp, in which the vesicles and the fHbp are both derived from the same host cells. The composition can also be made from vesicles and fHbp that are derived from different host cells so that the vesicles and fHbp are admixed after their separate purification.

Where desired (e.g., where the strains used to produce vesicles are associated with endotoxin or particular high levels of endotoxin), the vesicles are optionally treated to reduce endotoxin, e.g., to reduce toxicity following administration. 35 Although less desirable as discussed below, reduction of endotoxin can be accomplished by extraction with a suitable detergent (for example, BRIJ-96, sodium deoxycholate, sodium lauroylsarcosinate, EMPIGEN BB, TRITON X-100, TWEEN 20 (sorbitan monolaurate polyoxyethylene), 40 TWEEN 80, at a concentration of 0.1-10%, preferably 0.5-2%, and SDS). Where detergent extraction is used, it is preferable to use a detergent other than deoxycholate.

The vesicles of the antigenic compositions can be prepared without detergent, e.g., without use of deoxycholate. 45 Although detergent treatment is useful to remove endotoxin activity, it may deplete the native fHbp lipoprotein and/or chimeric fHbp (including lipidated chimeric fHbp) by extraction during vesicle production. Thus it may be particularly desirable to decrease endotoxin activity using technology that 50 does not require a detergent. In one approach, strains that are relatively low producers of endotoxin (lipopolysaccharide, LPS) are used so as to avoid the need to remove endotoxin from the final preparation prior to use in humans. For example, the vesicles can be prepared from *Neisseria* mutants 55 in which lipooligosaccharide or other antigens that may be undesirable in a vaccine (e.g. Rmp) is reduced or eliminated.

Vesicles can be prepared from *N. meningitidis* strains that contain genetic modifications that result in decreased or no detectable toxic activity of lipid A. For example, such strain 60 can be genetically modified in lipid A biosynthesis (Steeghs et al. Infect Immun 1999; 67:4988-93; van der Ley et al. Infect Immun 2001; 69:5981-90; Steeghs et al. J Endotoxin Res 2004; 10:113-9; Fissha et al, Infect Immun 73:4070, 2005). The immunogenic compositions may be detoxified by modification of LPS, such as downregulation and/or inactivation of the enzymes encoded by 1pxL1 or 1pxL2, respectively. Pro-

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duction of a penta-acylated lipid A made in 1pxL1 mutants indicates that the enzyme encoded by 1pxL1 adds the C12 to the N-linked 3-OH C14 at the 2' position of GlcN II. The major lipid A species found in 1pxL2 mutants is tetra-acylated, indicating the enzyme encoded by 1pxL2 adds the other C12, i.e., to the N-linked 3-OH C14 at the 2 position of GlcN I. Mutations resulting in a decreased (or no) expression of these genes (or decreased or no activity of the products of these genes) result in altered toxic activity of lipid A (van der Ley et al. 2001; 69:5981-90). Tetra-acylated (1pxL2 mutant) and penta acylated (1pxL1 mutant) lipid A are less toxic than the wild-type lipid A. Mutations in the lipid A 4'-kinase encoding gene (1pxK) also decrease the toxic activity of lipid A. Of particular interest for use in production of vesicles (e.g., MV or OMV) are N. meningitidis strains genetically modified so as to provide for decreased or no detectable functional LpxL1-encoded protein. Such vesicles provide for reduced toxicity as compared to N. meningitidis strains that are wildtype for LPS production, while retaining immunogenicity of chimeric fHbp.

LPS toxic activity can also be altered by introducing mutations in genes/loci involved in polymyxin B resistance (such resistance has been correlated with addition of aminoarabinose on the 4' phosphate of lipid A). These genes/loci could be pmrE that encodes a UDP-glucose dehydrogenase, or a region of antimicrobial peptide-resistance genes common to many enterobacteriaciae which could be involved in aminoarabinose synthesis and transfer. The gene pmrF that is present in this region encodes a dolicol-phosphate manosyl transferase (Gunn J. S., Kheng, B. L., Krueger J., Kim K., Guo L., Hackett M., Miller S. I. 1998. Mol. Microbiol. 27: 1171-1182).

Mutations in the PhoP-PhoQ regulatory system, which is a phospho-relay two component regulatory system (e.g., PhoP constitutive phenotype, PhoPc), or low Mg++ environmental or culture conditions (that activate the PhoP-PhoQ regulatory system) lead to the addition of aminoarabinose on the 4'-phosphate and 2-hydroxymyristate replacing myristate (hydroxylation of myristate). This modified lipid A displays reduced ability to stimulate E-selectin expression by human endothelial cells and TNF secretion from human monocytes.

Polymyxin B resistant strains are also suitable for use, as such strains have been shown to have reduced LPS toxicity (see, e.g., van der Ley et al. 1994. In: Proceedings of the ninth international pathogenic *Neisseria* conference. The Guildhall, Winchester, England). Alternatively, synthetic peptides that mimic the binding activity of polymyxin B may be added to the antigenic compositions to reduce LPS toxic activity (see, e.g., Rustici et al. 1993, Science 259:361-365; Porro et al. Prog Clin Biol Res. 1998; 397:315-25).

Endotoxin can also be reduced through selection of culture conditions. For example, culturing the strain in a growth medium containing 0.1 mg-100 mg of aminoarabinose per liter medium provides for reduced lipid toxicity (see, e.g., WO 02/097646).

Formulations

"Antigen composition", "antigenic composition" or "immunogenic composition" is used herein as a matter of convenience to refer generically to compositions comprising a chimeric fHbp as disclosed herein, which chimeric fHbp may be optionally conjugated to further enhance immunogenicity. Compositions useful for eliciting antibodies, particularly antibodies against *Neisseria meningitidis* group B (NmB), in a human are specifically contemplated by the present disclosure. Antigenic compositions can contain 2 or

more different chimeric fHbps, where the chimeric fHbps may present epitopes from different combinations of fHbp variants and/or subvariants.

Antigenic compositions generally comprise an immunologically effective amount of chimeric fHbp, and may further 5 include other compatible components, as needed. By "immunologically effective amount" is meant that the administration of that amount to an individual, either in a single dose, as part of a series of the same or different antigenic compositions, is effective to elicit an antibody response effective for treatment or prevention of a symptom of, or disease caused by, for example, infection by Neisseria, particularly N. meningitidis, more particularly Group B N. meningitidis. This amount varies depending upon the health and physical condition of the individual to be treated, age, the capacity of the 15 individual's immune system to produce antibodies, the degree of protection desired, the formulation of the vaccine, the treating clinician's assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through 20

Dosage regimen may be a single dose schedule or a multiple dose schedule (e.g., including booster doses) with a unit dosage form of the antigenic composition administered at different times. The term "unit dosage form," as used herein, 25 refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of the antigenic compositions of the present disclosure in an amount sufficient to produce the desired effect, which compositions are provided in associa- 30 tion with a pharmaceutically acceptable excipient (e.g., pharmaceutically acceptable diluent, carrier or vehicle). The antigenic composition may be administered in conjunction with other immunoregulatory agents.

Antigenic compositions can be provided in a pharmaceu- 35 tically acceptable excipient, which can be a solution such as a sterile aqueous solution, often a saline solution, or they can be provided in powder form. Such excipients can be substantially inert, if desired.

vant. Examples of known suitable adjuvants that can be used in humans include, but are not necessarily limited to, alum, aluminum phosphate, aluminum hydroxide, MF59 (4.3% w/v squalene, 0.5% w/v TWEEN 80<sup>TM</sup>, 0.5% w/v SPAN 85), CpG-containing nucleic acid (where the cytosine is unmethy-45 lated), QS21, MPL, 3DMPL, extracts from Aquilla, ISCOMS, LT/CT mutants, poly(D,L-lactide-co-glycolide) (PLG) microparticles, Quil A, interleukins, and the like. For experimental animals, one can use Freund's, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor- 50 muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dip-almitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE), and RIBI, which contains three components extracted 55 from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/ TWEEN 80 emulsion. The effectiveness of an adjuvant may be determined by measuring the amount of antibodies directed against the immunogenic antigen or antigenic 60 epitope thereof.

Further exemplary adjuvants to enhance effectiveness of the composition include, but are not limited to: (1) oil-inwater emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (see 65 below) or bacterial cell wall components), such as for example (a) MF59<sup>TM</sup> (WO 90/14837; Chapter 10 in Vaccine

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design: the subunit and adjuvant approach, eds. Powell & Newman, Plenum Press 1995), containing 5% Squalene, 0.5% TWEEN 80, and 0.5% SPAN 85 (optionally containing MTP-PE) formulated into submicron particles using a microfluidizer, (b) SAF, containing 10% Squalane, 0.4% TWEEN 80, 5% PLURONIC-blocked polymer L121, and thr-MDP either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, and (c) RIBI™ adjuvant system (RAS), (Ribi Immunochem, Hamilton, Mont.) containing 2% Squalene, 0.2% TWEEN 80, and one or more bacterial cell wall components such as monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL+CWS (DETOXTM); (2) saponin adjuvants, such as QS21 or STIMULON<sup>TM</sup> (Cambridge Bioscience, Worcester, Mass.) may be used or particles generated therefrom such as ISCOMs (immunostimulating complexes), which ISCOMS may be devoid of additional detergent e.g. WO 00/07621; (3) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (4) cytokines, such as interleukins (e.g. IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12 (WO99/44636), etc.), interferons (e.g. gamma interferon), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), etc.; (5) monophosphoryl lipid A (MPL) or 3-O-deacylated MPL (3dMPL) e.g. GB-2220221, EP-A-0689454, optionally in the substantial absence of alum when used with pneumococcal saccharides e.g. WO 00/56358; (6) combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions e.g. EP-A-0835318, EP-A-0735898, EP-A-0761231; (7) oligonucleotides comprising CpG motifs (Krieg Vaccine 2000, 19, 618-622; Krieg Curr opin Mol Ther2001 3:15-24; Roman et al., Nat. Med, 1997, 3, 849-854; Weiner et al., PNAS USA, 1997, 94, 10833-10837; Davis et al, J. Immunol, 1998, 160, 810-876; Chu et al., J. Exp. Med, 1997, 186, 1623-1631; Lipford et al, Ear. J. Immunol., 1997, 27, 2340-2344; Moldoveami e/ al., Vaccine, 1988, 16, 1216-1224, Krieg et al., Nature, 1995, 374, 546-549; Klinman et al., PNAS USA, 1996, 93, 2879-2883; Ballas et al, J. Immunol, 1996, 157, 1840-1845; Cowdery et al, J. Immunol, 1996, 156, The antigenic compositions can further comprise an adju- 40 4570-4575; Halpern et al, Cell Immunol, 1996, 167, 72-78; Yamamoto et al, Jpn. J. Cancer Res., 1988, 79, 866-873; Stacey et al, J. Immunol., 1996, 157, 2116-2122; Messina et al, J. Immunol, 1991, 147, 1759-1764; Yi et al, J. Immunol, 1996, 157, 4918-4925; Yi et al, J. Immunol, 1996, 157, 5394-5402; Yi et al, J. Immunol, 1998, 160, 4755-4761; and Yi et al, J. Immunol, 1998, 160, 5898-5906; International patent applications WO 96/02555, WO 98/16247, WO 98/18810, WO 98/40100, WO 98/55495, WO 98/37919 and WO 98/52581, i.e. containing at least one CG dinucleotide, where the cytosine is unmethylated; (8) a polyoxyethylene ether or a polyoxyethylene ester e.g. WO 99/52549; (9) a polyoxyethylene sorbitan ester surfactant in combination with an octoxynol (WO 01/21207) or a polyoxyethylene alkyl ether or ester surfactant in combination with at least one additional nonionic surfactant such as an octoxynol (WO 01/21152); (10) a saponin and an immunostimulatory oligonucleotide (e.g. a CpG oligonucleotide) (WO 00/62800); (11) an immunostimulant and a particle of metal salt e.g. WO 00/23105; (12) a saponin and an oil-in-water emulsion e.g. WO 99/11241; (13) a saponin (e.g. QS21)+3dMPL+IM2 (optionally+a sterol) e.g. WO 98/57659; (14) other substances that act as immunostimulating agents to enhance the efficacy of the composition. Muramyl peptides include N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-25 acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutarninyl-L-alanine-2-(1'-2'dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-

ethylamine MTP-PE), etc. Adjuvants suitable fo administration to a human are of particular interest.

The antigen compositions may comprise other components, such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium, carbonate, and the like. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like.

The concentration of chimeric fHbp in a formulation can vary widely (e.g., from less than about 0.1%, usually at or at least about 2% to as much as 20% to 50% or more by weight) and will usually be selected primarily based on fluid volumes, viscosities, and patient-based factors in accordance with the particular mode of administration selected and the patient's needs.

Chimeric fHBP-containing formulations can be provided in the form of a solution, suspension, tablet, pill, capsule, powder, gel, cream, lotion, ointment, aerosol or the like. It is recognized that oral administration can require protection of the compositions from digestion. This is typically accomplished either by association of the composition with an agent that renders it resistant to acidic and enzymatic hydrolysis or by packaging the composition in an appropriately resistant carrier. Means of protecting from digestion are well known in the art

Chimeric fHbp-containing formulations can also be provided so as to enhance serum half-life of chimeric fHBP following administration. For example, where isolated chimeric fHbp are formulated for injection, the chimeric fHbp may be provided in a liposome formulation, prepared as a 35 colloid, or other conventional techniques for extending serum half-life. A variety of methods are available for preparing liposomes, as described in, e.g., Szoka et al., Ann Rev. Biophys. Bioeng. 9:467 (1980), U.S. Pat. Nos. 4,235,871, 4,501, 728 and 4,837,028. The preparations may also be provided in 40 controlled release or slow-release forms. Immunization

The chimeric fHbp-containing antigenic compositions are generally administered to a human subject that is at risk of acquiring a *Neisserial* disease so as to prevent or at least 45 partially arrest the development of disease and its complications. An amount adequate to accomplish this is defined as a "therapeutically effective dose." Amounts effective for therapeutic use will depend on, e.g., the antigenic composition, the manner of administration, the weight and general state of 56 health of the patient, and the judgment of the prescribing physician. Single or multiple doses of the antigenic compositions may be administered depending on the dosage and frequency required and tolerated by the patient, and route of administration.

The chimeric fHbp-containing antigenic compositions are generally administered in an amount effective to elicit an immune response, particularly a humoral immune response, in the host. As noted above, amounts for immunization will vary, and can generally range from about 1  $\mu g$  to  $100 \, \mu g$  per 70  $\,^6$ 60 kg patient, usually 5  $\mu g$  to  $50 \, \mu g/70$  kg. Substantially higher dosages (e.g. 10 mg to 100 mg or more) may be suitable in oral, nasal, or topical administration routes. The initial administration can be followed by booster immunization of the same of different chimeric fHbp-containing antigenic  $\,^6$ 5 composition. Usually vaccination involves at least one booster, more usually two boosters.

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In general immunization can be accomplished by administration by any suitable route, including administration of the composition orally, nasally, nasopharyngeally, parenterally, enterically, gastrically, topically, transdermally, subcutaneously, intramuscularly, in tablet, solid, powdered, liquid, aerosol form, locally or systemically, with or without added excipients. Actual methods for preparing parenterally administrable compositions will be known or apparent to those skilled in the art and are described in more detail in such publications as Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pa. (1980).

An anti-chimeric fHbp immune response can be assessed by known methods (e.g. by obtaining serum from the individual before and after the initial immunization, and demonstrating a change in the individual's immune status, for example an immunoprecipitation assay, or an ELISA, or a bactericidal assay, or a Western blot, or flow cytometric assay, or the like).

The antigenic compositions can be administered to a human subject that is immunologically naive with respect to *Neisseria meningitidis*. In a particular embodiment, the subject is a human child about five years or younger, and preferably about two years old or younger, and the antigenic compositions are administered at any one or more of the following times: two weeks, one month, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 months, or one year or 15, 18, or 21 months after birth, or at 2, 3, 4, or 5 years of age.

It may be generally desirable to initiate immunization prior to the first sign of disease symptoms, or at the first sign of possible or actual exposure to infection or disease (e.g., due to exposure or infection by *Neisseria*).

### ATCC Deposit

Hybridomas producing the JAR 4, JAR 5, JAR 11, and JAR 32 monoclonal antibodies were deposited under the terms of the Budapest Treaty with the American Type Culture Collection, 10801 University Blvd., Manassas, Va. 20110-2209, USA (ATCC) on the date indicated in the table below, and were assigned the designations set out in the table below.

	ATCC Deposit No. (Deposit Date)	Material Deposited
	PTA-8943 (Feb. 7, 2008)	Hybridoma producing JAR 4 Monoclonal Antibody
5	PTA-8941 (Feb. 7, 2008)	Hybridoma producing JAR 5 Monoclonal Antibody
	PTA-8940 (Feb. 7, 2008)	Hybridoma producing JAR 10 Monoclonal Antibody
	PTA-8938 (Feb. 7, 2008)	Hybridoma producing JAR 11 Monoclonal Antibody
0	PTA-8942 (Feb. 7, 2008)	Hybridoma producing JAR 32 Monoclonal Antibody
	PTA-8939 (Feb. 7, 2008)	Hybridoma producing JAR 33 Monoclonal Antibody

It should be noted that JAR 5 mAb specifically binds to an epitope that at least overlaps with the epitope specifically bound by JAR 3 mAb, and that JAR 32 mAb specifically binds to an epitope that at least overlaps with the epitope specifically bounds by JAR 35 mAb.

These deposits were made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations there under (Budapest Treaty). This assures maintenance of a viable culture of the deposit for 30 years from the date of deposit and for at least five (5) years after the most recent request for the furnishing of a sample of the deposit received by the depository. The deposits will be

made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Children's Hospital & Research Center at Oakland and the ATCC (the assignee of the present application) which assures that all restrictions imposed by the depositor on the availability to the 5 public of the deposited material will be irrevocably removed upon the granting of the pertinent U.S. patent, assures permanent and unrestricted availability of the progeny of the culture of the deposit to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign 10 patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 U.S.C. §122 and the Commissioner's rules pursuant thereto (including 37 C.F.R. §1.14 with particular 15 reference to 886 OG 638).

The assignee(s) of the present application has agreed that if a culture of the materials on deposit should die or be lost or destroyed when cultivated under suitable conditions, the materials will be promptly replaced on notification with 20 another of the same. Availability of the deposited material is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

#### **EXAMPLES**

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

Materials and Methods

The following methods and materials were used in the Examples below.

fHbp Sequencing.

The fHbp gene was amplified from genomic DNA pre- 40 pared with the DNeasy Tissue kit (Qiagen, Valencia, Calif.) by polymerase chain reaction using primers A1 and B2 and cycling parameters described previously (Masignani et al. J Exp Med 2003, 197:789-99). The PCR products were purified using QiaQuick PCR purification kit (Qiagen) and were 45 eluted in 30 µl of sterile deionized H<sub>2</sub>O. The fHbp DNA sequences were determined by a commercial sequencing facility using the primers A1 and 22 described previously (Masignani et al., 2003).

Source of Data.

Protein sequences analyzed in the first study were encoded by 69 fHbp genes from *Neisseria meningitidis* case isolates from the United States (Beernink et al. J Infect Dis 2007, 195:1472-9), Europe (Beernink and Granoff Infect Immun 2008, 76:2568-2575; Beernink et al. Infect Immun 2008, 55 76:4232-4240) and Africa (Beernink et al., *J Infect Dis* 2009, doi:10.1086/597806). This data set included 48 sequences determined as part of our previous studies and 21 new sequences performed for the present study. 95 additional fHbp gene sequences were obtained from Genbank ("www" followed by ".ncbi.nlm.nih" followed by ".gov") by performing translated BLAST (tblastn) searches with fHbp amino acid sequences from strains MC58 (variant 1/sub-family B) and M1239 (variant 3/sub-family A). Among these 164 nucleotide sequences from our collection and Genbank, which 65 included three genome sequences (Parkhill et al. Nature 2000, 404:502-506; Peng et al. Genomics 2008, 91:78-87;

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Tettelin et al. Science 2000, 287:1809-1815), fHbp genes that encoded 63 unique protein sequences were identified. These 63, plus 6 additional unique fHbp amino acid sequences obtained from the Neisseria.org fHbp peptide database (neisseria.org), were used for the analysis of 69 unique fHbp peptides. The respective Genbank accession numbers and/or peptide identification numbers and the characteristics of the source strains are listed in FIG. 1A-1G (Table 7).

Thirty-eight (55%) of the 69 peptides were classified in the variant 1 group of Masignani et al. 2003, 15 (22%) in the variant 2 group, and 16 (23%) in the variant 3 group. Of the 69 source strains, one was capsular group A, 57 were group B, seven were group C, two were group W-135, and two were group X. Multi-locus sequence type (MLST) information was available for 58 of the strains of which 15 were from the ST-269 clonal complex, 12 each were from the ST-11, 10 were from the ST-41/44 complexes, four were from the ST-162, five were from the ST-213 complexes, three each were from the ST-8 and ST-32 complexes. Six strains were from other clonal complexes and had sequence types ST-4, ST-35, ST-751, ST-4821, ST-5403 and ST-6874. The 11 strains without MLST information were not available for testing.

In a second study, the dataset further included 172 additional distinctive sequences that were subsequently added to the Neisseria.org database ("http" followed by "://neisseria.org/perl/agdbnet/agdbnet.pl?file=nm\_fhbp" followed by ".xml") as of November 2009. In describing the 242 unique proteins (FIGS. 10A-10I, Table 9), the protein identification (ID) numbers from the peptide database at the Neisseria.org website were employed.

A combination of approaches for analysis of complete or partial protein sequences was used. Sequences were aligned with MUSCLE (EBI, v3.7, "ebi.ac" followed by ".uk/Tools/ muscle/index" followed by ".html") (Edgar R C. (2004) Nucleic Acids Res. 32:1792-7; Edgar R C (2004) BMC Bioinform. 5:113) configured for highest accuracy. The accuracy of the alignments was confirmed by visual inspection and using the program JALVIEW (Water house A M et al. (2009) Bioinformatics 25:1189-91). Alignments also were performed on the individual modular variable segments between the blocks of invariant residues. Networks were generated using SplitsTree, version 4.0 (Huson D H et al., (2006) Mol Biol Evol 23:254-67), with default parameters. Statistical tests for branch support were performed using the bootstrapping method (1000 replicates).

Phylogenic Analysis.

The analysis of complete or partial protein sequences was 50 performed on the platform at "www" followed by ".phylogeny" followed by ".fr" (Dereeper et al. Nucleic Acids Res 2008, 36:W465-469) and comprised the following steps. Sequences were aligned with MUSCLE (v3.7) (Edgar Nucleic Acids Res 2004, 32:1792-1797) configured for highest accuracy. The respective alignments, which contained up to three sites of insertions or deletions, were inspected and verified by adjacent invariant sequences. The phylogenic tree was reconstructed using the maximum likelihood method implemented in the PhyML program (v3.0 aLRT). Reliability for internal branch was assessed using the bootstrapping method (100 bootstrap replicates). Phylograms were displayed with MEGA 4.0 (Tamura et al. Mol Biol Evol 2007, 24:1596-1599). The phylograms were rooted on peptide 1 for consistency. The percent sequence identities within and between variable segment types of sequences were determined using ClustalW (Larkin et al. Bioinformatics 2007, 23:2947-2948).

Cloning, Expression and Purification of Recombinant Proteins.

Expression plasmids were constructed by PCR amplification of fHbp genes from genomic DNA as described previously (Masignani et al. (2003) *J Exp Med* 197:789-99). The 5 genes encoded fHbp ID 1 (Modular group I), 28 (group II), 22 (group III), 15 (group IV), 79 (group V) and 77 (group VI). C-terminal hexahistidine-tagged recombinant fHbps were expressed in *Escherichia coli* BL21(DE3) (Novagen, Madison, Wis., US), and purified as described elsewhere (Beernink 10 P T et al. (2008) *Infect Immun* 76:2568-75).

Mouse Antisera.

Groups of five-week-old CD-1 female mice (10 mice per group) were obtained from Charles River (Wilmington, Md., US). The mice were immunized via the intraperitoneal route 15 (IP) with three doses of vaccine given at 3-week intervals. Each 100-µl dose contained 20 µg of recombinant protein mixed with Freund's adjuvant (FA) (Sigma-Aldrich, St. Louis, Mo., US). (Complete FA for the first dose and incomplete for subsequent doses). Terminal blood samples were 20 obtained three weeks after the last dose. The animal procedures were performed under a protocol approved by the Institutional Animal Care and Use Committee of the Children's Hospital Oakland Research Institute.

Bacterial Strains.

Characteristics of the *N. meningitidis* strains used to measure serum bactericidal activity are summarized in Tables 7 and 9. Two isolates each was selected from modular groups I to VI. For five of the modular groups, one strain from each pair was a low expresser of fHbp and the other a higher 30 expresser as measured as described below. For modular group V, only strains that expressed intermediate quantities of fHbp were identified and, therefore, used for the assays.

Complement-Dependent Serum Bactericidal Antibody Activity.

The bactericidal assay was performed as described elsewhere, using early log phase bacteria grown for approximately 2 h in Mueller-Hinton broth (BD Biosciences, Franklin Lakes, N.J., US) supplemented with 0.25% glucose (w/v) and 0.02 mM cytidine 5'-monophospho-N-acetylneuraminic 40 acid (Sigma-Aldrich, St, Louis, Mo., US) [11]. The complement source was serum from a non-immune healthy adult with normal hemolytic complement activity. The serum was passed over a protein G SEPHAROSE column (HI-TRAP Protein G HP, GE Healthcare, Piscataway, N.J., US) to 45 remove IgG antibodies (Beernink PT et al. (2009) *J Infect Dis* 199:1360-8).

Quantitative Western Blotting of fHbp.

N. meningitidis cells were grown in broth cultures to early log phase, heat-killed (56° C. for 1 h), collected by centrifu- 50 gation, and resuspended in PBS to an optical density of 0.6. Proteins in the heat-killed cells  $(1-4\times10^7 \text{ CFU})$  were separated by SDS-PAGE using 4-12% NuPAGE gels (Invitrogen, Carlsbad, Calif., US) as specified by the manufacturer, and were transferred to PVDF membranes (Immobilon-FL; Mil- 55 lipore, Billerica, Mass., US) using a Western blot module (Invitrogen, Carlsbad Calif., US). After blocking overnight at 4° C. in blocking buffer (Li-Cor Biosciences, Lincoln, Nebr., US), fHbp modular groups I and IV (variant 1 group) were detected with anti-fHbp JAR 5 or, JAR 31, for modular groups 60 II, III, V or VI (variant groups 2 or 3) (Beernink P T et la. (2008) Infect Immun 76:4243-40). The secondary antibody was goat anti-mouse IgG-IRDye800CW (1:10,000, Rockland Immunochemicals, Gilbertsville, Pa., US).

To determine the quantity of bound protein, the membranes 65 were scanned at 800 nm wavelength using an infrared scanner (Li-Cor Odyssey, Lincoln, Nebr., US), and the integrated

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intensities of the bands were calculated with software provided by the manufacturer (version 3.0.21). The results of testing purified, recombinant fHbp from each of the six modular groups indicated that the IR signal intensities were proportional to the  $\log_{10}$  of the quantities loaded in the range of 0.03 to 2  $\mu$ g (representative data for control proteins in modular groups I and VI are shown in FIG. 11). Representative expression data are shown for two strains with fHbp in modular group I, and two strains in modular group VI (FIG. 11).

Statistical Analysis.

Statistical calculations were performed using Prism 5 for Mac OSX, version 5.0a (GraphPad Software, La Jolla, Calif., US). The proportion of isolates with fHbp in different modular groups in each country was computed along with the respective 95 percent confidence intervals calculated from the binomial distribution. Differences found in the proportion of isolates in the respective modular groups were compared by Fisher Exact Test (two-tailed) or chi-square analyses.

#### OVERVIEW OF EXAMPLES

Based on previous analyses of the sequences of the mature peptide, fHbp was classified into two sub-families (Fletcher et al. Infect Immun 2004, 72:2088-2100) or three variant groups (Masignani et al., 2003) (FIG. 3). Analysis presented herein indicated that the overall architecture of meningococcal fHbp contains a limited number of specific modular combinations of five variable segments, with each segment corresponding to one of two types, designated as  $\alpha$  or  $\beta$ , based on sequence similarity to the respective segments of fHbp peptides 1 (variant 1 group) or 28 (variant 3 group). As described in the Examples section below and shown in FIG. 4, the amino acid sequences of two N. gonorrhoeae orthologs had architectures corresponding to natural chimeras in modular group V (FIG. 8). The propensity for *Neisseria* to transfer genes horizontally (Hotopp et al. Microbiology 2006, 152:3733-3749), coupled with the similar modular architectures of meningococcal and gonococcal fHbp peptides described here, suggests that during evolution, the respective progenitor genes recombined at conserved sequences encoding the invariant blocks of residues hypothesized to be points of junction.

Among the 69 distinct meningococcal fHbp peptides, 65 (94%) could be assigned to one of six modular groups (FIG. 8). Nearly 60% of the peptides were in modular groups I or II, which comprised only  $\alpha$  or  $\beta$  type segments, respectively. The remaining peptides were natural chimeras of  $\alpha$  and  $\beta$  segments. Modular fHbp groups, III, IV, and V (FIG. 8), could each have been generated from the two progenitor types by a single recombination event. The modular fHbp group VI could have been generated by two recombination events. Similarly the four exceptional sequences described in the Examples section could each have been generated through recombination at loci encoding alternative blocks of other conserved residues. For a protein comprising five segments, each of which can be of one of two types, there are  $2^5=32$ theoretical independent modular combinations. That we identified only six modular groups suggests that there are functional or structural constraints on the molecule that select for certain combinations of the modules found in the prevalent fHbp modular groups.

fHbp structural data are available from two recent NMR studies (Cantini et al., *J Biol Chem* 2009, doi:10.1074/jbc.C800214200; Mascioni et al. *J Biol Chem* 2009, doi: 10.1074/jbc.M808831200) and a crystallographic study of fHbp in a complex with a complement regulatory protein

(Cantini et al., 2009; Mascioni et al., 2009; Schneider et al. *Nature* 2009, doi:10.1038/nature07769). In the crystal structure, Schneider et al reported that binding to residues located on the short consensus repeat region 6 (SCR 6) of fH was mediated by charged amino acid residues in the fHbp structure that mimicked portions of sugar molecules known to bind to fH (Schneider et al. 2009). Analysis as presented in FIG. 9 indicated that these fH contact residues were located on three of the five variable segments identified in the present study. Of the 21 fH contact residues, five were invariant among the 69 unique fHbp peptides analyzed in our study and ten other contact residues were conserved.

All three fHbp variable segments with fH contact residues also contained amino acids that affected epitopes recognized by bactericidal murine anti-fHbp mAbs (FIG. 9). The central locations of the fH contact residues in the amino- or carboxylterminal domains appeared to be distinct from the peripheral locations of the residues affecting epitope expression. This reciprocal relationship suggests that the fHbp structures in contact with fH infrequently elicited bactericidal antibodies.

One limitation of fHbp as a vaccine candidate is antigenic variability (Beernink & Granoff, 2009; Masignani et al., 2003). Thus, serum antibodies to recombinant fHbp in the  $^{25}$ variant 1 group were bactericidal primarily only against strains with variant 1 proteins (Beernink et al. 2007; Fletcher et al. Infect Immun 2004, 72; 2088-2100; Masignani et al. 2003), while antibodies to fHbp in the variant 2 or 3 groups had activity primarily against strains with homologous variant 2 or 3 proteins but had no activity against strains with fHbp in the variant 1 group. These observations suggest that epitopes in the C and E segments of fHbp, for which variant 1 proteins were phylogenetically separated from those of 35 variant 2 and 3 proteins (FIGS. 6 and 7), are more important for eliciting bactericidal antibodies than those in the A, B or D segments, where variant 1 proteins clustered together with variant 2 and/or 3 proteins (FIGS. 5 and 6). However, all of the variable segments with the exception of segment B contained residues previously identified as affecting epitopes recognized by murine bactericidal anti-fHbp mAbs (Beernink et al. 2008; Beernink et al. Mol Immunol 2009b, doi:10.1016/ j.molimm.2009.02.021; Guiliani et al Infect Immun 2005, 45 73:1151-1160). Further, some of the mAbs inhibited binding of fH to the bacterial surface (Madico et al. J Immunol 2006, 177:501-510), and some combinations of mAbs that individually were not bactericidal elicited cooperative bactericidal activity (Beernink et al. 2008; Beernink et al. 2009b; Welsch et al. J Infect Dis 2008, 197:1053-1061). The latter included mAb JAR 4, which was specific for an epitope on variable segment A (Beernink et al. 2009b), and elicited cooperative bactericidal activity with second mAbs with epitopes 55 on variable segment C (for example JAR 3 or 5) or variable segment E (for example, JAR 13 or mAb502).

To minimize the possibility of fHbp escape mutants, an ideal meningococcal vaccine should include antigenic targets other than fHbp (Giuliani et al., 2006). However, there may be limits to the number of recombinant proteins that can be combined in a multicomponent vaccine because of the large dose of total protein required, poor tolerability, or loss of immunogenicity of the individual components. To decrease the number of individual fHbp components, we engineered chimeric fHbp molecules containing the amino-terminal

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domain of a variant 1 fHbp with the carboxyl-terminal domain of a variant 2 protein using G136 as the junctional point (Beernink & Granoff, 2008). The resulting chimeric protein expressed epitopes from all three variant groups, and the chimeric fHbp vaccines elicited serum antibodies with bactericidal activity against a panel of genetically diverse strains expressing fHbp variant 1, 2 or 3. The junctional point selected for creation of the recombinant chimeric vaccines was located in an invariant portion of the middle of the variable C segment, which, in the present study, was not identified as a junctional point for any of the natural fHbp chimeric proteins described here. The use of natural junctional points that flank the C segment may result in improved recombinant chimeric fHbp vaccines that elicit broad bactericidal antibodies than other vaccines. It may also be possible to introduce amino acid mutations in one or more of the variable segments to introduce epitopes from heterologous fHbp variants (Beernink & Granoff 2008; Beernink et al. 2009b). Thus, the presence of such fH-binding epitopes in the chimeric fHbp polypeptides can provide for production of antibodies that can facilitate protection against N. meningitidis.

In conclusion, the analysis herein shows that the overall architecture of fHbp can be defined by a limited number of specific modular combinations of variable segments flanked by invariant sequences. Collectively, the data suggest that recombination occurred between *N. meningitidis* and *N. gonorrhoeae* progenitor sequences to generate an antigenically diverse family of meningococcal factor H-binding proteins. The data presented in the Examples thus provide insights into the evolution of fHbp variants, and provide a rational basis for classification of groups of peptide variants. Population-based surveillance studies will be required to define the prevalence of fHbp from different modular groups among strains causing carriage and/or disease, and the relationship between the fHbp modular group and other strain characteristics such as capsular group, clonal complex and PorA variable regions.

Details of the studies that led to this discovery are set out below.

### Example 1

# Phylogenic Analysis

69 unique fHbp amino acid sequences were aligned to generate a phylogram shown in FIG. 3. For each sequence, the peptide identification number assigned in fHbp peptide database at neisseria.org is shown and, if known, the multi-locus sequence type (MLST) clonal complex is shown in parentheses. The lower left branch shows variant group 1 as defined by Masignani et al 2003 (sub-family B of Fletcher et al 2004); sub-family A contained two branches, variant groups 2 and 3. Prototype peptides for each of the variant groups 1, 2 and 3 are indicated with arrows and labeled with the name of the source strain of the respective genes. The phylogram was constructed by multiple sequence alignment as described in Methods. The scale bar shown at the bottom indicates 5 amino acid changes per 100 residues.

The analysis showed the two major branches previously designated as sub-families A and B (Fletcher et al. 2004). Sub-family A contained fHbp sequences in antigenic variant groups 2 and 3, and sub-family B corresponded to fHbps in

the antigenic variant group 1 (Masignani et al. 2003). For some clonal complexes, there were examples of strains with fHbp in each of the variant groups (for example, for ST-11, peptides 2, 3, 6, 9, 10, 11 and 78 in the variant 1 group, peptides 17, 22, 23 and 27 in the variant 2 group and peptide 59 in the variant 3 group). The strains from the ST-32 clonal complex had fHbp in two of the variant groups (peptides 1 and 89 in the variant 1 group and peptide 76 in the variant 3 group) and the ST-8 clonal complex had fHbp only in one variant group (peptides 16, 50, and 77 in the variant 2 group). The distribution of fHbp variants in all of the observed clonal complexes is given in Table 2 below. Peptide IDs listed are assigned by the fHbp peptide database at neisseria.org.

TABLE 2

Distribution of factor H binding protein variants in each clonal complex.									
Clonal	Number in Variant group (peptide ID)								
Complex	Variant 1	Variant 2	Variant 3	Total	_				
ST-4	1 (5)	0	0	1	_				
ST-8	0	3 (16, 50, 77)	0	3					
ST-11	7 (2, 3, 6, 9, 10, 11, 78)	4 (17, 22, 23, 27)	1 (59)	12					
ST-32	2 (1, 89)	0	1 (76)	3					
ST-35	1 (7)	0	0 `	1					
ST-41/44	6 (4, 8, 12, 14, 86, 87)	2 (19, 20)	2 (28, 84)	10					
ST-162	1 (88)	1 (21)	2 (29, 82)	4					
ST-213	1 (90)	1 (24)	3 (30, 31, 85)	5					
ST-269	10 (13, 15, 60-63, 65, 66, 69, 71)	2 (68, 83)	3 (64, 70, 72)	15					
ST-4821	1 (80)	0	0	1					
Unassigned	2 (73, 74)	0	1 (79)	3					
Not done	6 (40, 54-58)	2 (25, 49)	3 (45-47)	11					

## Example 2

# The Architecture of fHbp is Modular

Blocks of two to five invariant residues were identified that separate the proteins into segments of variable residues. See FIG. 4 for detail. The invariant residues are shown as vertical 45 rectangles. The top three panels show representative architectures of three *N. meningitidis* fHbp variants in groups 1, 2 and 3 (peptide ids, 1, 16 and 28, respectively). Each of the three variants had repetitive amino-terminal elements (N-term), and variable segments A through E, which in some cases differed in length among the variants. The amino acid positions of the last residue in each variable segment are shown.

With the exception of the amino-terminal element, there were identical respective invariant amino acid sequences and homologous variable segments in two *N. gonorrhoeae* orthologs (Genbank accession numbers AE004969 and CP001050), which overall had 96% amino acid sequence identity with meningococcal fHbp peptide 79 (variant 3 meningococcal proteins). In the first study, all 69 fHbp sequence variants had blocks of 2 to 5 invariant residues that flanked five modular variable segments (FIG. 4). These invariant residues were also conserved in 170 of the 172 additional fHbp variants analyzed in study 2. One exception, protein ID 65 33, had SHFDF (SEQ ID NO: 279) instead of SRFDF (SEQ ID NO: 3) between the A and B segments, and the other

exception, protein, ID 150, had IEHLE (SEQ ID NO:6) instead of IEHLK (SEQ ID NO: 5) between the D and A segments (FIG. 4).

The blocks of invariant residues appeared to represent natural junctional points for recombination of the respective genes encoding the meningococcal and gonococcal proteins. The phylogeny of each the variable segments defined by these junctional points was analyzed. In the analysis of the 69 distinctive meningococcal fHbp peptide variants described below, the numbering of the amino acid residues is based on the mature fHbp peptide 1 encoded by a gene from MC58 in the variant 1 group (Masignani et al. 2003). The numbering for the respective variant 2 and 3 proteins differ from that of the variant 1 protein by -1 and +7, respectively (FIG. 4).

### Example 3

## The Amino-Terminal Repetitive Element

For all 69 sequences, the mature fHbp began with a cysteine residue that is lipidated by signal peptidase II, which was followed by three invariant amino acid residues, SSG. This invariant sequence was followed by a repetitive variable sequence consisting of 1 to 6 glycine and/or serine residues and then by two invariant glycine residues (FIG. 4). The variable portion of the amino-terminal element consisted of a single glycine residue for 34 of the 69 peptides (nearly all in the variant 1 group, Table 3 shown below), or GG residues for 12 of the peptides (all in the variant 2 group). The other common variable sequence was GGGSGG (SEQ ID NO: 9) (8 in the variant 1 group and 7 in the variant 3 group).

TABLE 3

Variable	Number of Peptides in Variant Group							
Sequence <sup>1</sup>	Variant 1	Variant 2	Variant 3					
G	29	3	2					
GG	0	12	0					
SGG	0	0	1					
GGGS	0	0	2					
(SEQ ID NO: 1)								
SGSGG	0	0	1					
(SEQ ID NO: 2)								
GGGSGG	8	0	6					
(SEQ ID NO: 3)								
GGGSGS	1	0	4					
(SEQ ID NO: 4)								

<sup>&</sup>lt;sup>1</sup>The mature fHbp begins with an invariant tetrapeptide, CSSG (SEQ ID NO: 2), which is followed by a repetitive variable sequence consisting of 1 to 6 glycine and/or serine residues, which is then followed by two invariant glycine residues.

## Example 4

# Downstream Variable Segments

There were five variable segments, which we designated A, B, C, D or E (FIG. 4). Based on the presence of certain signature amino acid residues and sequence similarity (Table 4), each of the five segments could be segregated into one of two types. One of the types had signature amino acid residues and sequence similarity to peptides in the antigenic variant 1 group. The other type had signature amino acid residues and sequence similarity to peptides in the antigenic variant 3 group, which also were similar to those of the gonococcal ortholog (see FIG. 4). Also see Table 4 presented below.

TABLE 4

	Amino acid identity within and between sequence groups by segment.											
			α Seqı	лепсе Т	ypes	β Seque	Identity					
Variable Segment		Invariant Block <sup>2</sup>	Signature residues <sup>3</sup>	No. <sup>4</sup>	Identity Range (%)	Signature residues <sup>3</sup>	No.4	Identity Range (%) <sup>4</sup>	between $\alpha$ and $\beta$ Types $(\%)^4$			
	0.72	GDEDE7	OGT	40	00.100	DOI LEDNI	2.1	00.1005	60.705			
A	8-73	SRFDF <sup>7</sup>	QSV	48	89-100	DSI and KDN	21	89-100 <sup>5</sup>	69-78 <sup>5</sup>			
В	79-93	GEFQ <sup>8</sup>	IRQ	53	80-100 <sup>5</sup>	VQK	16	100	60-66 <sup>5</sup>			
C	98-159	$^{\mathrm{DD}}$	QDS	38	85-100	NNP	31	93-100	32-45			
D	162-180	IEHLK <sup>9</sup>	AG or AS	58	89-100	PN	11	84-100	52-63			
E	186-253	$KQ^6$	SLGI	38	86-100	$HLAL^{10}$	31	94-100 <sup>5</sup>	48-57 <sup>5</sup>			

<sup>&</sup>lt;sup>1</sup>Amino acid numbering based on the mature fHbp from strain MC58.

For purposes of classification, the first group of segments is designated as  $\alpha$  types and the second group as  $\beta$  types. The amino acid identity of the respective segments within an  $\alpha$  or  $\beta$  type ranges from 80 to 100% (Table 4). In contrast, the identity of segments between types ranged from 69-78% for segment A, which was located in a conserved region of the N-terminal portion of the molecule, to 32-45% in the C segment, which encompassed resides 98-159 in a much less conserved region of the protein (Table 4). For each segment, distinct sequence variants were assigned a unique identifier beginning with a letter, A through E, to represent the variable segment; followed by an  $\alpha$  or  $\beta$  to indicate the presence of residues with the respective types described above, followed by a number for each distinct sequence (listed in FIG. 2A-2E (Table 8)).

Segment A began at amino acid residue 8 immediately after the invariant GG sequence and extended to position 73 (Table 4). Among the 69 fHbp variants, segment A contained 16 distinctive  $\alpha$  sequence variants, and 9 distinctive  $\beta$  sequence variants (FIG. 5). Where multiple peptides possessed an identical sequence in a segment, the number of peptides is given in parentheses. The scale bar indicates 5 amino acid changes per 100 residues. Based on the phylogram, the A segments of most of the fHbps in the variant 1 group (shown in FIG. 5) clustered with those in the variant 2 group, whereas the A segments of fHbps in the variant 3 group were in a separate cluster. The most common α variant (N=14) was designated A. $\alpha$ .1, which was present in 4 fHbp peptides in the variant 1 group and 10 in the variant 2 group. The most common  $\beta$ variant was designated A.β.1, which was present in 6 peptides, all in the variant 3 group.

Segment B began at position 79 immediately after an invariant SRFDF (SEQ ID NO: 3) sequence and extended to position 93 (Table 4). Among the 69 fHbp peptides, segment 60 B contained 7 different  $\alpha$  variant sequences and a single  $\beta$  sequence (FIG. 5). The phylogenic analysis showed that the B segments of fHbp peptides in the variant 1 and 2 groups clustered together (FIG. 5), which were distinct from those in the variant 3 group. The most common B segment, B. $\alpha$ .1, was 65 present in 40 fHbp peptides (28 in the variant 1 group and 12 in the variant 2 group).

In contrast to the A and B segments, the respective C (residues 98-159) and E (residues 186-253) segments of fHbp in the variant 2 and 3 groups clustered together and were separated from those of fHbps in the variant 1 group 1 (FIG. 6). All of the D segments (residues 162-180) of strains with fHbp in the variant group 1 were  $\alpha$  types while those of fHbp peptides in the variant 2 or 3 groups were  $\alpha$  or  $\beta$  (FIG. 7). The most common D segment, D. $\alpha$ .1 (N=39), was present in 21 fHbp v.1 peptides, 9 v.2 peptides and 9 v.3 peptides.

## Example 5

# Classification of fHbp Variants by Modular Groups

Based on the phylogenic analysis of the variable segments of fHbp described above, the majority of these different fHbp variants studied were categorized into six distinct fHbp modular groups (I to VI) (FIG. 8). Forty of the 69 fHbp peptides (58%) comprised only  $\alpha$  (N=33) or  $\beta$  (N=7) type segments, which were designated fHbp modular groups I and II, respectively (FIG. 8). The  $\alpha$  segments are shown in gray and the  $\beta$  segments are shown in white. The remaining 29 peptides (42%) could be classified into one of four chimeras derived from recombination of different  $\alpha$  or  $\beta$  segments (designated fHbp modular groups III, IV, V or VI; N=25) or, as described below, were other chimeras (N=4) in which one of the five segments used an exceptional junctional position at other conserved blocks of sequences. As seen in FIG. 6, a representative peptide in each modular group is indicated by the peptide identification number from the fHbp database at neisseria.org. The number of unique sequences observed within each fHbp modular group is indicated at the right. This analysis excluded four peptides sequences with exceptional junctional points at one of their segments.

Although the amino-terminal repetitive segment described above was not used to define the modular fHbp groups, the majority (88 to 100%) of modular group I, III and VI peptides sequences had amino-terminal repetitive elements of 1 or 2 glycine residues (Table 3). In contrast, the majority (75 to 100%) of the peptides in modular groups II, IV and V had amino-terminal elements of 3 to 6 glycine and serine residues. The length of this amino-terminal element may affect the

<sup>&</sup>lt;sup>2</sup>Invariant block of residues immediately following each variable segment. The invariant sequence immediately before segment A was GG (See FIG. 4).

GG (See FIG. 4).

See FIG. 2A-2E: (Table 8) for complete sequences of each variable segment.

<sup>&</sup>lt;sup>4</sup>Number of sequences in each sequence type for each variable segment (N = 61 peptides).
<sup>5</sup>Four Htlp sequences with exceptional junctional positions at other conserved blocks of residues were excluded from the percent

identity analyses (see text).

<sup>6</sup>Corresponds to carboxyl-terminal end of fHbp sequence

<sup>&#</sup>x27;SEQ ID NO: 3

<sup>8</sup>SEQ ID NO: 4

<sup>9</sup>SEO ID NO: 5

<sup>10</sup>SEQ ID NO: 280

<sup>11</sup> SEQ ID NO: 281

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distance of the protein from the bacterial membrane and surface-accessibility of certain epitopes.

In the second study, all but three of the 172 new fHbp variants could be classified into one of the six modular groups (I to VI, FIG. 4, Panel A). The three exceptions (protein ID 207, 67 and 175) had distinctive combinations of modular segments derived from  $\alpha$  or  $\beta$  type lineages and were assigned to one of three new modular groups (VII, VIII and IX, respectively).

Of 242 distinct fHbp variants in the expanded database,  $^{10}$  125 proteins were in modular group I (FIG. 4), which contained only  $\alpha$  segments, and 20 proteins were in modular group II (entirely  $\beta$  segments). The remaining 97 fHbp sequence variants were natural chimeras of  $\alpha$  and  $\beta$  segments and were assigned to modular groups III through IX (together,  $^{15}$  40 percent of all variants). FIG. 4 shows the fHbp variant groups as described by Masignani et al. (2003) J Exp Med 1997:789-99 for each of the modular groups. The variant groups were assigned based on the relatedness of the overall amino acid sequences of the respective proteins.

## Example 6

# Chimeric fHbp Peptides Containing Junction Points within a Variable Segment

Four fHbp sequences had modular structures similar to those described above except that one of the junctional points between two of the segments utilized alternative invariant sequences. For example, fHbp peptide 55 was similar to pep- 30 tides in modular group IV (FIG. 8) except that the A segment switched from a  $\beta$  type sequence to an  $\alpha$  type sequence at an invariant AQGAE (SEQ ID NO: 278) starting at residue 50 rather than at SRFDF (SEQ ID NO: 3) starting at residue 74. This A segment was designated A.β.9 (β because of its higher 35 sequence identity to other  $\beta$  type A segments than to the  $\alpha$ type A segments; FIG. 5). Two other fHbp peptides, 24 and 25 (FIG. 2A-2E (Table 8)), had modular structures similar to peptides in modular group III (FIG. 8) but had B segments that switched from an  $\alpha$  type to a  $\beta$  type sequence at an 40 invariant IEV beginning at residue 82 instead of GEFQ (SEQ ID NO: 4 at position 94. This exceptional B segment, designated B. $\alpha$ .3, was categorized as an  $\alpha$  type because of its higher sequence identity to other  $\alpha$  type B segments than to the  $\beta$  type B segments (FIG. 5). The fourth exceptional fHbp 45 modular structure, peptide 82 (FIG. 2A-2E (Table 8)), was similar to type V, except that its E segment, designated E. $\beta$ . 10, switched from an  $\alpha$  type sequence to a  $\beta$  type at residue A196 instead of at IEHLK (SEQ ID NO: 5) starting at position 181 (FIG. 6).

## Example 7

# Structural Features of the Variable and Invariant fHbp Segments

The respective variable and invariant segments of fHbp were mapped onto a molecular model based on the published coordinates from the fHbp crystal structure (FIG. 9) (Schneider et al. 2009). The amino- and carboxyl-termini, 60 labeled N and C, respectively, in panel A are in identical positions in panels B and C. The figure was constructed with PyMol (pymol.org). The models in the center of panels A, B and C have been rotated 180 degrees on the Y-axis from the corresponding models on the far left, while the models on the 65 far right have been rotated 90 degrees on the X-axis as compared with the models in the middle.

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The ribbon models in panel A show the two previously described major domains of fHbp (Cantini et al. 2009; Mascioni et al. 2009; Schneider et al. 2009), each containing independently folded beta-structures, which are connected together by a structured, four amino acid residue linker located in variable segment C. The amino-terminal domain, which begins at residue 8 and extends to residue 136, includes variable segments A and B and part of C (note that the first 14 residues of the mature protein are not present in the crystal structure). The carboxyl-terminal domain, which extends from residue 141 to 255, includes the carboxyl-terminal portion of the C and the entire D and E variable segments.

Panel B shows the corresponding space-filled models. The amino acid residues previously reported to be in contact with fH based on the structure of the fHbp-fH complex are depicted in black. These residues form clusters on variable segments A, C and E and are visible in the models shown in the middle and far right of panel B. Since fH is known to bind 20 to fHbp on live bacteria (Madico et al. 2006; Schneider et la. 2006), this binding site must be surface-exposed. The invariant blocks of residues that flank each of the five variable segments, are localized on the opposite face of the protein and shown in white in panels B and C (far left model). Since the amino-terminus, which contains the signal anchor, extends from this face of the protein (bottom surface of model at far right, panel B), the invariant residues are predicted to be located entirely on the surface of the molecule anchored to the cell wall. The presence of these invariant sequences on the membrane-associated surface suggests that there are structural constraints, perhaps a requirement for a partner protein, for anchoring and/or orienting fHbp on the bacterial cell membrane.

Previous studies mapped the epitopes of eleven bactericidal anti-fHbp mAbs (Beernink et al. 2008; Beernink et al. 2009b; Giuliani et al. 2005; Scarselli et al J Mol Biol 2009, 386:97-108). In panel C (FIG. 9), the amino acids affecting expression of each of these epitopes are depicted in black and the previously defined fH contact residues (shown in black as in panel B). With the exception of segment B, all of the variable segments contained epitopes recognized by bactericidal mAbs. The amino acids affecting the epitopes generally were located on the periphery of the amino- and carboxylterminal domains of fHbp whereas the residues in contact with fH were located in clusters in the central portions of each of the two domains. The epitopes of certain mAbs such as JAR 3, 5 or 13, which were previously reported to inhibit binding of fH to fHbp (Beernink et al. 2008; Beernink et al. 2009b), involved amino acids located in proximity to some of 50 the fH contact residues. However, there was no example of overlap between the two sets of residues.

### Example 8

## Frequency of fHbp Modular Groups Among Isolates Causing Disease in Different Countries

The analysis described above provided information on the extent of fHbp modular group diversity. For this following analysis, the fHbp sequence data from systematically collected group B isolates in the U.S. and Europe reported by Murphy et al. (2009) *J Infect Dis* 200:379-89 were used. The isolates were from cases in the United States between 2001 and 2005 (N=432), and from the United Kingdom (N=536), France (N=244), Norway (N=23) and the Czech Republic (N=27) for the years 2001 to 2006. The U.S. data were supplemented with fHbp sequences of 143 additional isolates that

had been systematically collected at multiple sites in the U.S. as part of another study (Beernink PT et al. (2007) *J Infect Dis* 195:1472-9).

Among the total of 1405 systematically collected group B isolates, modular group I was found in 59.7%, group II in 51.7%, group III in 8.1%, group IV in 10.6%, group V in 6.1%, and group VI in 13.6%. The new modular groups, VII, VIII and IX, were each found in  $\leq$ 0.1%. The respective distributions of the fHbp modular groups in the different countries as stratified by the variant group classification of Masignani et al. (2003)  $J Exp \ Med \ 197:789-99$  are shown in FIG. 12. For this analysis, the data from Norway and Czech Republic were excluded, since the number of isolates in each of these collections was too small for precise estimates of the respective frequencies of the modular groups. The respective percentages of the modular groups in the two U.S. collections are shown separately.

Isolates in variant 1 group of Masignani consisted of modular groups I, IV and VII. Modular group I strains, which have entirely  $\alpha$ -type segments, predominated in all three countries 20 (54 to 64 percent of all isolates). In the UK, however, 23% of all isolates were modular group IV, which are natural chimeras of  $\alpha$ - and  $\beta$ -type segments (FIG. 8), as compared with <1% in the two U.S. collections, and 3 percent of isolates from France (P<0.001 by chi square). Since there was only 25 one isolate in modular group VII (France), the frequency of this modular group is not shown in FIG. 12.

Isolates in variant 2 group included modular groups III and VI, which are all natural chimeras of  $\alpha$ - and  $\beta$ -type segments. Modular groups III or VI were present in approximately equal 30 proportions of isolates in the two U.S. collections, while modular group VI predominated among variant 2 isolates from the UK and France.

Isolates with variant 3 group included modular group II (entirely  $\beta$ -type segments) and modular groups V, VIII and 35 IX, which are chimeras. In France and the UK, modular group V accounted for the majority of the isolates with variant 3 fHbp, while in the two U.S. collections there were approximately equal numbers of modular group II or V proteins. Modular groups VIII and IX accounted for <0.1% of the 40 isolates. Because of the low percentages, these groups are not shown on FIG. 12.

# Example 9

Strain Susceptibility to Anti-fHbp Serum Bactericidal Activity in Relation to Modular Group and fHbp Expression

FIG. 13 depicts human complement bactericidal titers of 50 serum pools from mice immunized with recombinant fHbp vaccines representative of modular groups I-VI. The heights

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of the bars represent the respective median titers of each of the six antisera (3 to 4 pools per modular group) when tested against the specific test strain. For example, the upper left panel shows the data for strain H44/76, which is a high expresser of fHbp in modular group I (relative expression is designated by +++; actual values are shown in Table 5 below). The median bactericidal titer of the homologous anti-fHbp modular group I antiserum (black bar) was ~1:6000. The respective titers of the five heterologous anti-fHbp modular group antisera (white bars) against strain H44/76 were 1 to 2 log<sub>10</sub> lower, ranging from <1:10 (antisera to modular group II) to ~1:200 (antisera to modular group IV). The corresponding median titers of the anti-modular group II and IV antisera when tested against control strains with homologous fHbp modular groups II (strain SK104 in the Variant 3 panel) or IV (NM452 in the Variant 1 panel) were >1:2000. Thus, these and the other heterologous antisera had high antibody activity when measured against control strains with the respective homologous fHbp modular groups.

There were lower bactericidal titers against low fHbp expressing strains than against high expressing strains (compare respective graphs on the right side of FIG. 13 showing data for low expressing strains [designated +/-], with those on the left showing higher expressers). For example the antimodular group I antiserum had a titer of 1:6000 against H44/76, a high fHbp modular group I expressing strain, but a titer of ~1:100 against strain 03S-0408, a low fHbp modular group I expresser. (For modular group V, both test strains were intermediate expressers, since a pair of high and low fHbp expressing strains were not available).

There was a trend for lower cross-reactivity of anti-fHbp bactericidal activity against strains with low expression of fHbp from heterologous modular groups than for high expressing strains (see for example, data for low expressing strains 03S-0408 (modular group I), M01573 (modular group IV), and RM1090 (modular group III), which were killed only by their respective homologous modular group antisera as compared with the broader bactericidal activity against the respective higher fHbp expressing strains (H44/76, NM452, and 03S-0673; FIG. 13). Strain NMB (fHbp modular group VI) was completely resistant to anti-fHbp bactericidal activity (titer <1:10), which apparently was a result of low fHbp expression (Table 5). The amino acid sequence of the vaccine 45 used to prepare the anti-modular group VI antisera differed by a single amino acid from fHbp expressed by NMB, and the resulting antisera were bactericidal against a higher expressing strain (961-5945) with fHbp modular group VI. Further, the resistant strain, NMB, was killed by a control anti-PorA mAb at a concentration of 0.15 μg/ml, which was identical to that of an anti-PorA mAb required for killing of the anti-fHbp susceptible 961-5945 strain.

TABLE 5

Characteristics of <i>N. meningitidis</i> strains used for measuring serum bactericidal activity.											
Factor H-binding protein											
Strain name	sequence types	clonal complex	Variant group	Protein ID	Modular group	Expression (ng/10 <sup>6</sup> cells)	Genbank accession number				
H44/76	32	32	1	1	I	31	AY548370				
03S-0408	11	11	1	78	I	3	ACF35432				
SK104	5748	162	3	99	II	11	GQ219769				
M1239	437	41/44	3	28	II	2	ABF82029				
03S-0673	1364	32	2	23	III	14	GU056306				

Characteristics of N. meningitidis strains used for measuring serum
bactericidal activity.

			Factor H-binding protein						
Strain name	sequence types	clonal complex	Variant group	Protein ID	Modular group	Expression (ng/10 <sup>6</sup> cells)	Genbank accession number		
RM1090	11	11	2	22	III	2	ABY26518		
NM452	283	269	1	15	IV	20	ABL14232		
M01573	44	41/44	1	55	IV	1	AAR84481		
S3032	6874	n/a	3	79	V	8	ACH48234		
MD1475	162	162	3	82	V	7	GQ219772		
961-5945	153	8	2	16	VI	5	AAR84453		
NMB	8	8	2	50	VI	1	AY330379		

The antibodies used for the quantitative measurement of fHbp and the reactivity of various modular group proteins for each antibody is shown in the Table below.

TABLE 6

	TABLE 0										
					antibody rea				s		
An	JAR 3 or JAR 32 Anti-fHbp mAb JAR 1 JAR 4 JAR 5 JAR 10 JAR 11 JAR 13 JAR 31 JAR 35 JAR 33									JAR 33	
Epitope location	٤	mino acid idue(s)	R204	(25-27) YGN (57-59) KDN	G121, K122	K180 and E192 <sup>4</sup>	A174	S216	Unk <sup>6</sup>	K174	R180 and E192 <sup>4</sup>
		odular gment	Εα	Αα	Сα	$\begin{array}{c} \mathrm{D}\alpha \text{ and} \\ \mathrm{E}\beta \end{array}$	$D\alpha$	Εβ	$\mathrm{UD}^6$	Dβ	Dβ and Eβ
Modular Group	Variant Group	Protein ID No.		Reactivity of Recombinant Protein by ELISA <sup>5</sup>							
I	1	1	1	1	1	0	0	0	0	0	0
II	3	28	0	0	0	0	0	1	1	1	1
III	2	22	0	1	0	0	0	0	1	1	1
IV	1	15	0	0	1	0	0	0	0	0	0
V	3	79	0	0	O	1	1	1	1	0	0
VI	2	77	0	1	0	1	1	1	1	0	0

 $<sup>^{1}\</sup>mathrm{All}$  of the MAbs were bactericidal when tested in certain combinations with human complement

## SEQUENCE LISTING

<sup>&</sup>lt;sup>3</sup>Discontinuous epitope; presence of KDN suppresses epitope [1]

<sup>&</sup>lt;sup>4</sup>Discontinuous epitope requiring specific combinations of charged residues

 $<sup>^5</sup> Assigned~^{\circ}1"$  if Optical density >3 SD above background binding when tested at 1  $\mu g/ml,~^{\circ}0",$  negative.

<sup>&</sup>lt;sup>6</sup>UD, location of epitope undetermined

```
Thr Gly Lys Leu Lys Asn Asp Lys Val Ser Arg Phe Asp Phe Ile Arg
Gln Ile Glu Val Asp Gly Gln Leu Ile Thr Leu Glu Ser Gly Glu Phe
Gln Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Phe Gln Thr Glu
Gln Ile Gln Asp Ser Glu His Ser Gly Lys Met Val Ala Lys Arg Gln
Phe Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu
Pro Glu Gly Gly Arg Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser Asp
Asp Ala Gly Gly Lys Leu Thr Tyr Thr Ile Asp Phe Ala Ala Lys Gln
Gly Asn Gly Lys Ile Glu His Leu Lys Ser Pro Glu Leu Asn Val Asp
Leu Ala Ala Asp Ile Lys Pro Asp Gly Lys Arg His Ala Val Ile
                          200
Ser Gly Ser Val Leu Tyr Asn Gln Ala Glu Lys Gly Ser Tyr Ser Leu
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 \hbox{Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Glu } \\
Thr Tyr Gly Asn Gly Asp Ser Leu Asp Thr Gly Lys Leu Lys Asn Asp
Lys Val
65
<210> SEQ ID NO 21
<211> LENGTH: 66
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 21
Val Ala Ala Asp Ile Gly Thr Gly Leu Ala Asp Ala Leu Thr Ala Pro
Leu Asp His Lys Asp Lys Gly Leu Gln Ser Leu Thr Leu Asp Gln Ser 20 \phantom{\bigg|}25\phantom{\bigg|}
Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp
Lys Val
<210> SEQ ID NO 22
<211> LENGTH: 66
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 22
Val Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Ala Pro
                5
Leu Asp His Lys Asp Lys Gly Leu Gln Ser Leu Thr Leu Asp Gln Ser
```

-continued

30 Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys 40 Thr Tyr Gly Asn Gly Asp Arg Leu Asn Thr Gly Lys Leu Lys Asn Asp Lys Val <210> SEQ ID NO 23 <211> LENGTH: 66 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <223> OTHER INFORMATION: fHbp sequence variant <400> SEQUENCE: 23 Val Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Ala Pro 1  $\phantom{-}$  5  $\phantom{-}$  10  $\phantom{-}$  15 Phe Asp His Lys Asp Lys Gly Leu Gln Ser Leu Thr Leu Asp Gln Ser 20  $\phantom{\bigg|}25\phantom{\bigg|}$  30 Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Glu 35  $\phantom{\bigg|}40\phantom{\bigg|}40\phantom{\bigg|}45\phantom{\bigg|}$ Ile Tyr Gly Asn Gly Asp Ser Leu Asp Thr Gly Lys Leu Lys Asn Asp 50 60 Lys Val 65 <210> SEQ ID NO 24 <211> LENGTH: 66 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: fHbp sequence variant <400> SEQUENCE: 24 Val Thr Ala Asp Ile Gly Thr Gly Leu Ala Asp Ala Leu Thr Ala Pro Leu Asp His Lys Asp Lys Gly Leu Gln Ser Leu Thr Leu Asp Gln Ser  $20 \\ 25 \\ 30 \\$ Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp Lys Ile <210> SEQ ID NO 25 <211> LENGTH: 66 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: fHbp sequence variant <400> SEQUENCE: 25 Val Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Ala Pro Leu Asp His Lys Asp Lys Gly Leu Gln Ser Leu Thr Leu Asp Gln Ser Val Arg Lys Lys Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys

```
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp
Lys Val
<210> SEQ ID NO 26
<211> LENGTH: 66
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 26
Val Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Ala Pro
Leu Asp His Lys Asp Lys Gly Leu Arg Ser Leu Thr Leu Asp Gln Ser 20 \phantom{\bigg|}25\phantom{\bigg|} 30
Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp
Lys Val
65
<210> SEQ ID NO 27
<211> LENGTH: 66
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 27
Val Ala Ala Asp Ile Gly Ala Val Leu Ala Asp Ala Leu Thr Ala Pro
                                  10
Leu Asp His Lys Asp Lys Ser Leu Gln Ser Leu Thr Leu Asp Gln Ser
Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp
Lys Val
<210> SEQ ID NO 28
<211> LENGTH: 66
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 28
Val Ala Ala Asp Ile Gly Thr Gly Leu Ala Asp Ala Leu Thr Ala Pro
Leu Asp His Lys Asp Lys Gly Leu Arg Ser Leu Thr Leu Asp Gln Ser
                                25
Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys
                         40
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp
                        55
```

```
Lys Val
65
<210> SEQ ID NO 29
<211> LENGTH: 66
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 29
Val Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Ala Pro
Leu Asp His Lys Asp Lys Gly Leu Gln Ser Leu Thr Leu Asp Gln Ser 20 \phantom{\bigg|}25\phantom{\bigg|} 30
Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Val Glu Lys _{
m 35} _{
m 40} _{
m 45}
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp 50 \\
Lys Val
65
<210> SEQ ID NO 30
<211> LENGTH: 66
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 30
Val Ala Val Asp Ile Gly Thr Gly Leu Ala Asp Ala Leu Thr Ala Pro 1 \phantom{\bigg|} 10 \phantom{\bigg|} 15
Leu Asp His Lys Asp Lys Gly Leu Gln Ser Leu Thr Leu Asp Gln Ser 20 $25$
Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp
Lys Ile
<210> SEQ ID NO 31
<211> LENGTH: 66
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 31
Val Ala Ala Asp Ile Gly Thr Gly Leu Ala Asp Ala Leu Thr Ala Pro 1 \phantom{\bigg|} 10 \phantom{\bigg|} 15
Leu Asp His Lys Asp Lys Gly Leu Gln Ser Leu Thr Leu Asp Gln Ser
Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Glu
                                40
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp 50 \\
Lys Val
65
```

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<210> SEQ ID NO 32
<211> LENGTH: 66
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 32
Val Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Ala Pro
Leu Asp His Lys Asp Lys Gly Leu Gln Ser Leu Ile Leu Asp Gln Ser 20 \phantom{\bigg|}25\phantom{\bigg|} 30
Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys _{\mbox{35}}
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp
Lys Val
<210> SEQ ID NO 33
<211> LENGTH: 66
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 33
Val Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Ala Pro
                             10
Leu Asp His Lys Asp Lys Gly Leu Gln Ser Leu Thr Leu Asp Gln Ser
Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp
Lys Ile
<210> SEQ ID NO 34
<211> LENGTH: 66
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 34
Val Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Ala Pro
Leu Asp His Lys Asp Lys Gly Leu Gln Ser Leu Thr Leu Asp Gln Ser 20 \phantom{\bigg|}25\phantom{\bigg|}
Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Ser Lys Leu Lys Asn Asp
Lys Val
<210> SEQ ID NO 35
<211> LENGTH: 66
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
```

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<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 35
Val Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Val Pro
Leu Asp His Lys Asp Lys Ser Leu Gln Ser Leu Thr Leu Asp Gln Ser 20 25 30
Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp
Lys Val
<210> SEQ ID NO 36
<211> LENGTH: 66
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 36
Val Ala Ala Asp Ile Gly Thr Gly Leu Ala Asp Ala Leu Thr Ala Pro 1 \phantom{\bigg|} 5 \phantom{\bigg|} 10 \phantom{\bigg|} 15
Leu Asp His Lys Asp Lys Ser Leu Gln Ser Leu Thr Leu Asp Gln Ser 20 $25$
Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys _{
m 35} _{
m 40} _{
m 45}
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp
Lys Val
<210> SEQ ID NO 37
<211> LENGTH: 66
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 37
Val Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Thr Pro 1 \phantom{\bigg|} 10 \phantom{\bigg|} 15
Leu Asp His Lys Asp Lys Ser Leu Gln Ser Leu Thr Leu Asp Gln Ser 25 \phantom{\bigg|}25
Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys _{
m 35} _{
m 40} _{
m 45}
Thr Tyr Gly Asn Gly Asp Asn Leu Asn Thr Gly Lys Leu Lys Asn Asp
Lys Val
<210> SEQ ID NO 38
<211> LENGTH: 66
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 38
```

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Val Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Ala Pro 1 \phantom{\bigg|} 5 \phantom{\bigg|} 10 \phantom{\bigg|} 15
Leu Asp His Lys Asp Lys Gly Leu Gln Ser Leu Ala Leu Asp Gln Ser 20 \phantom{-}25\phantom{+}\phantom{0} 30
Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp
Lys Val
<210> SEQ ID NO 39
<211> LENGTH: 66
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 39
Val Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Ala Pro 1 \phantom{\bigg|} 5 \phantom{\bigg|} 10 \phantom{\bigg|} 15
Leu Asp His Lys Asp Lys Gly Leu Gln Ser Leu Thr Leu Asp Gln Ser
Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Glu 35 40 45
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp
Lys Val
<210> SEQ ID NO 40
<211> LENGTH: 66
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 40
Val Thr Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Ala Pro
Leu Asp His Lys Asp Lys Gly Leu Arg Ser Leu Met Leu Asp Gln Ser 20 \phantom{\bigg|}25\phantom{\bigg|} 30
Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys _{\mbox{35}}
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp
Lys Ile
<210> SEQ ID NO 41
<211> LENGTH: 66
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 41
Val Ala Ala Asp Ile Gly Val Gly Leu Ala Asp Ala Leu Thr Thr Pro
```

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Leu Asp His Lys Asp Lys Gly Leu Gln Ser Leu Thr Leu Asp Gln Ser
Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp
Lys Val
<210> SEQ ID NO 42
<211> LENGTH: 65
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 42
Val Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Ala Pro
                                10
Leu Asp His Lys Asp Lys Gly Leu Gln Ser Leu Thr Leu Asp Gln Ser 20 $25$
Val Arg Lys Asn Glu Lys Leu Lys Leu Ser Ala Gln Gly Ala Glu Lys
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp
Lys
65
<210> SEQ ID NO 43
<211> LENGTH: 66
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 43
Val Ala Ala Asp Ile Gly Thr Gly Leu Ala Asp Ala Leu Thr Ala Pro
1
Leu Asp His Lys Asp Lys Gly Leu Gln Ser Leu Thr Leu Asp Gln Ser
Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp
Lys Ile
<210> SEQ ID NO 44
<211> LENGTH: 66
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 44
Val Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Ala Pro
Leu Asp His Lys Asp Lys Gly Leu Gln Ser Leu Thr Leu Asp Gln Ser
Val Arg Lys Asn Glu Lys Leu Lys Leu Ser Ala Gln Gly Ala Glu Lys
```

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45
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp
                          55
Lys Val
<210> SEQ ID NO 45
<211> LENGTH: 66
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 45
Val Ala Ala Asp Ile Gly Thr Gly Leu Ala Asp Ala Leu Thr Ala Pro
Leu Asp His Lys Asp Lys Gly Leu Gln Ser Leu Met Leu Asp Gln Ser 20 \phantom{\bigg|}25\phantom{\bigg|} 30
Val Arg Lys Asn Glu Lys Leu Lys Leu Ser Ala Gln Gly Ala Glu Lys _{\rm 35} _{\rm 40} _{\rm 45}
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp 50 \\
Lys Ile
65
<210> SEQ ID NO 46
<211> LENGTH: 66
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 46
Val Thr Ala Asp Ile Gly Thr Gly Leu Ala Asp Ala Leu Thr Ala Pro
Leu Asp His Lys Asp Lys Gly Leu Gln Ser Leu Thr Leu Asp Gln Ser 20 \\ 25 \\ 30
Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp
Lys Val
<210> SEQ ID NO 47
<211> LENGTH: 66
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 47
Val Ala Ala Asp Ile Gly Thr Gly Leu Ala Asp Ala Leu Thr Ala Leu
                                     10
Pro Asp His Lys Asp Lys Gly Leu Gln Ser Leu Thr Leu Asp Gln Ser
Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys
                              40
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp
```

```
Lys Val
<210> SEQ ID NO 48
<211> LENGTH: 66
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 48
Val Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Ala Pro
Val Arg Lys Lys Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys 35 \ \ 40 \ \ 45
Thr Tyr Gly Asn Gly Asp Ser Leu Ser Thr Gly Lys Leu Lys Asn Asp
Lys Val
65
<210> SEQ ID NO 49
<211> LENGTH: 66
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 49
Val Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Ala Pro
                                  10
Leu Asp His Lys Asp Lys Gly Leu Gln Ser Leu Thr Leu Asp Gln Ser
                             25
Val Arg Arg Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp
Lys Val
<210> SEQ ID NO 50
<211> LENGTH: 66
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 50
Val Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Ala Pro
                                 10
Leu Asp His Lys Asp Lys Ser Leu Gln Ser Leu Thr Leu Asp Gln Ser
Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp
Lys Ile
```

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<210> SEQ ID NO 51
<211> LENGTH: 66
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 51
Val Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Ala Pro
Leu Asp His Lys Asp Lys Gly Leu Gln Ser Leu Met Leu Asp Gln Ser
Val Arg Lys Asn Glu Lys Leu Lys Leu Ser Ala Gln Gly Ala Glu Lys _{\rm 35} _{\rm 40} _{\rm 45}
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp 50 \\
Lys Val
<210> SEQ ID NO 52
<211> LENGTH: 67
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 52
Val Ala Ala Asp Ile Gly Thr Gly Leu Ala Tyr Ala Leu Thr Ala Pro
Leu Asp His Lys Asp Lys Ser Leu Gln Ser Leu Thr Leu Asp Gln Ser 20 \\ 25 \\ 30 \\
Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Gln Thr Gly Lys Leu Lys Asn
Asp Lys Val
65
<210> SEQ ID NO 53
<211> LENGTH: 66
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 53
Val Thr Ala Asp Ile Gly Thr Gly Leu Ala Asp Ala Leu Thr Ala Pro 1 \phantom{\bigg|} 10 \phantom{\bigg|} 15
Leu Asp His Lys Asp Lys Gly Leu Gln Ser Leu Met Leu Asp Gln Ser
Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp
Lys Ile
<210> SEQ ID NO 54
<211> LENGTH: 66
<212> TYPE: PRT
```

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<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 54
Val Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Ala Pro
Leu Asp His Lys Asp Lys Gly Leu Gln Ser Leu Met Leu Asp Gln Ser
Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys _{\mbox{35}}
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp
Lys Ile
<210> SEQ ID NO 55
<211> LENGTH: 66
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 55
Val Ala Ala Asp Ile Gly Thr Gly Leu Ala Asp Ala Leu Thr Ala Pro
Leu Asp Tyr Lys Asp Lys Gly Leu Gln Ser Leu Met Leu Asp Gln Ser
Val Arg Lys Asn Glu Lys Leu Lys Leu Ser Ala Gln Gly Ala Glu Lys
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp 50 60
Lys Val
<210> SEQ ID NO 56
<211> LENGTH: 66
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 56
Val Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Ala Pro
Leu Asp Tyr Lys Asp Lys Gly Leu Gln Ser Leu Thr Leu Asp Gln Ser
Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp
Lys Val
65
<210> SEQ ID NO 57
<211> LENGTH: 66
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
```

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<400> SEQUENCE: 57
Val Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Ala Pro
                                     10
Leu Asp His Lys Asp Lys Gly Leu Gln Ser Leu Thr Leu Asp Gln Ser
Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Glu
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Asp Lys Leu Lys Asn Asp
Lys Val
<210> SEQ ID NO 58
<211> LENGTH: 66
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 58
Val Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Val Leu Thr Ala Pro
Leu Asp His Lys Asp Lys Gly Leu Gln Ser Leu Thr Leu Asp Gln Ser 20 \phantom{\bigg|}25\phantom{\bigg|} 30
Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp 50 \\
Lys Val
65
<210> SEQ ID NO 59
<211> LENGTH: 66
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 59
Val Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Thr Pro
Leu Asp His Lys Asp Lys Gly Leu Arg Ser Leu Met Leu Asp Gln Ser
Val Arg Lys Asn Glu Lys Leu Lys Leu Ser Ala Gln Gly Ala Glu Lys
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp 50 60
Lys Val
65
<210> SEQ ID NO 60
<211> LENGTH: 66
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 60
Val Val Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Ala Pro
                                   10
```

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Leu Asp His Lys Asp Lys Gly Leu Gln Ser Leu Met Leu Asp Gln Ser
Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys
Thr Tyr Gly Asn Gly Asp Ile Leu Asn Thr Gly Lys Leu Lys Asn Asp
Lys Val
<210> SEQ ID NO 61
<211> LENGTH: 69
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 61
Leu Asp His Lys Asp Lys Gly Leu Gln Ser Leu Thr Leu Asp Gln Ser 20 $25$
Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys
Thr Phe Lys Ala Gly Asp Lys Asp Asn Ser Leu Asn Thr Gly Lys Leu
Lys Asn Asp Lys Ile
<210> SEQ ID NO 62
<211> LENGTH: 66
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 62
Ile Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Ala Pro
Leu Asp His Lys Asp Lys Ser Leu Gln Ser Leu Thr Leu Asp Gln Ser
Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp
Lys Val
<210> SEQ ID NO 63
<211> LENGTH: 66
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 63
Val Ala Ala Asn Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Ala Pro
                        10
Leu Asp His Lys Asp Lys Ser Leu Gln Ser Leu Thr Leu Asp Gln Ser
```

20 25 30

```
Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp
Lys Val
65
<210> SEQ ID NO 64
<211> LENGTH: 66
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 64
Val Ala Ala Asp Ile Gly Ala Gly Leu Thr Asp Ala Leu Thr Ala Pro
Leu Asp His Lys Asp Lys Ser Leu Gln Ser Leu Thr Leu Asp Gln Ser
                       25
Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys
                          40
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp 50 \\
Lys Val
65
<210> SEQ ID NO 65
<211> LENGTH: 66
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 65
Val Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Ala Pro
Leu Asp His Lys Asp Lys Gly Leu Gln Ser Leu Met Leu Asp Gln Ser
                               25
Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Glu
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp
Lys Val
<210> SEQ ID NO 66
<211> LENGTH: 66
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 66
Val Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Ala Pro
                                    1.0
Phe Asp His Lys Asp Lys Gly Leu Gln Ser Leu Thr Leu Asp Gln Ser
Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp
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50
                        55
                                            60
Lys Ile
65
<210> SEQ ID NO 67
<211> LENGTH: 69
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 67
Val Ala Ala Asp Ile Gly Thr Gly Leu Ala Asp Ala Leu Thr Ala Pro
Leu Asp His Lys Asp Lys Gly Leu Lys Ser Leu Thr Leu Glu Asp Ser
Ile Pro Gln Asn Gly Thr Leu Thr Leu Ser Ala Gln Gly Ala Glu Lys
Thr Phe Lys Ala Gly Asp Lys Asp Asn Ser Leu Asn Thr Gly Lys Leu 50 60
Lys Asn Asp Lys Ile
<210> SEQ ID NO 68
<211> LENGTH: 69
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 68
Val Ala Ala Asp Ile Gly Thr Gly Leu Ala Asp Ala Leu Thr Thr Pro
Leu Asp His Lys Asp Lys Gly Leu Lys Ser Leu Thr Leu Glu Asp Ser
Ile Pro Gln Asn Gly Thr Leu Thr Leu Ser Ala Gln Gly Ala Glu Lys
                         40
Thr Phe Lys Ala Gly Asp Lys Asp Asn Ser Leu Asn Thr Gly Lys Leu
Lys Asn Asp Lys Ile
<210> SEQ ID NO 69
<211> LENGTH: 69
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 69
Val Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Ala Pro
                                    10
Leu Asp His Lys Asp Lys Gly Leu Lys Ser Leu Thr Leu Glu Asp Ser
                              25
Ile Ser Gln Asn Gly Thr Leu Thr Leu Ser Ala Gln Gly Ala Glu Arg
                            40
Thr Phe Lys Ala Gly Asp Lys Asp Asn Ser Leu Asn Thr Gly Lys Leu
Lys Asn Asp Lys Ile
```

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<210> SEQ ID NO 70
<211> LENGTH: 69
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 70
Val Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Ala Pro
Leu Asp His Lys Asp Lys Gly Leu Lys Ser Leu Thr Leu Glu Asp Ser
Ile Ser Gln Asn Gly Thr Leu Thr Leu Ser Ala Gln Gly Ala Glu Lys
Thr Phe Lys Val Gly Asp Lys Asp Asn Ser Leu Asn Thr Gly Lys Leu
Lys Asn Asp Lys Ile
<210> SEQ ID NO 71
<211> LENGTH: 69
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 71
Val Ala Ala Asp Ile Gly Thr Gly Leu Ala Asp Ala Leu Thr Ala Pro
Leu Asp His Lys Asp Lys Gly Leu Lys Ser Leu Thr Leu Glu Asp Ser
                                25
Ile Ser Gln Asn Gly Thr Leu Thr Leu Ser Ala Gln Gly Ala Glu Lys
                        40
Thr Phe Lys Val Gly Asp Lys Asp Asn Ser Leu Asn Thr Gly Lys Leu
Lys Asn Asp Lys Ile
<210> SEQ ID NO 72
<211> LENGTH: 69
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 72
Val Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Ala Pro
Leu Asp His Lys Asp Lys Gly Leu Lys Ser Leu Thr Leu Glu Asp Ser
                               25
Ile Pro Gln Asn Gly Thr Leu Thr Leu Ser Ala Gln Gly Ala Glu Lys
Thr Phe Lys Ala Gly Gly Lys Asp Asn Ser Leu Asn Thr Gly Lys Leu
                       55
Lys Asn Asp Lys Ile
<210> SEQ ID NO 73
<211> LENGTH: 69
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEOUENCE: 73
Val Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Ala Pro
Leu Asp His Lys Asp Lys Gly Leu Lys Ser Leu Thr Leu Glu Asp Ser
Ile Pro Gln Asn Gly Thr Leu Thr Leu Ser Ala Gln Gly Ala Glu Lys
Thr Phe Lys Ala Gly Asp Lys Asp Asn Ser Leu Asn Thr Gly Lys Leu
Lys Asn Asp Lys Ile
<210> SEQ ID NO 74
<211> LENGTH: 69
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 74
Ile Ala Ala Asp Ile Gly Thr Gly Leu Ala Asp Ala Leu Thr Ala Pro 1 \phantom{\bigg|} 10 \phantom{\bigg|} 15
Leu Asp His Lys Asp Lys Gly Leu Lys Ser Leu Thr Leu Glu Asp Ser
Ile Pro Gln Asn Gly Thr Leu Thr Leu Ser Ala Gln Gly Ala Glu Lys
                           40
Thr Phe Lys Ala Gly Asp Lys Asp Asn Ser Leu Asn Thr Gly Lys Leu
Lys Asn Asp Lys Ile
<210> SEQ ID NO 75
<211> LENGTH: 66
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 75
Val Thr Ala Asp Ile Gly Thr Gly Leu Ala Asp Ala Leu Thr Ala Pro
Leu Asp His Lys Asp Lys Gly Leu Lys Ser Leu Thr Leu Glu Asp Ser
Ile Ser Gln Asn Gly Thr Leu Thr Leu Ser Ala Gln Gly Ala Glu Lys
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp
Lys Val
65
<210> SEQ ID NO 76
<211> LENGTH: 66
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
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<400> SEQUENCE: 76
Val Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Ala Pro
Leu Asp His Lys Asn Lys Gly Leu Lys Ser Leu Thr Leu Glu Asp Ser
Ile Pro Gln Asn Gly Thr Leu Thr Leu Ser Ala Gln Gly Ala Glu Lys
Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp
Lys Val
<210> SEQ ID NO 77
<211> LENGTH: 69
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 77
Val Ala Ala Asp Ile Gly Thr Gly Leu Ala Asp Ala Leu Thr Ala Pro
Leu Asp His Lys Asp Lys Gly Leu Lys Ser Leu Thr Leu Glu Asp Ser
                                25
Ile Pro Gln Asn Gly Thr Leu Thr Leu Ser Ala Gln Gly Ala Glu Lys
                          40
Thr Phe Lys Ala Gly Gly Lys Asp Asn Ser Leu Asn Thr Gly Lys Leu
Lys Asn Asp Lys Ile
<210> SEQ ID NO 78
<211> LENGTH: 69
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 78
Val Ala Ala Asp Ile Gly Thr Gly Leu Ala Asp Ala Leu Thr Ala Pro
Leu Asp His Lys Asp Lys Gly Leu Lys Ser Leu Thr Leu Glu Asp Ser
Ile Pro Gln Asn Gly Thr Leu Thr Leu Ser Ala Gln Gly Ala Glu Lys
Thr Phe Lys Val Gly Asp Lys Asp Asn Ser Leu Asn Thr Gly Lys Leu
Lys Asn Asp Lys Ile
<210> SEQ ID NO 79
<211> LENGTH: 69
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 79
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Val Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Thr Pro

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10
Leu Asp His Lys Asp Lys Gly Leu Lys Ser Leu Thr Leu Glu Asp Ser
                                  25
Ile Pro Gln Asn Gly Thr Leu Thr Leu Ser Ala Gln Gly Ala Glu Lys
Thr Phe Lys Ala Gly Asp Lys Asp Asn Ser Leu Asn Thr Gly Lys Leu
Lys Asn Asp Lys Ile
<210> SEQ ID NO 80
<211> LENGTH: 69
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 80
Val Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Ala Pro 1 \phantom{\bigg|} 5 \phantom{\bigg|} 10 \phantom{\bigg|} 15
Leu Asp His Lys Asp Lys Gly Leu Lys Ser Leu Thr Leu Glu Asp Ser 20 \phantom{\bigg|}25\phantom{\bigg|} 30
Ile Pro Gln Asn Gly Thr Leu Thr Leu Ser Ala Gln Gly Val Glu Lys
                             40
Thr Phe Lys Ala Gly Asp Lys Asp Asn Ser Leu Asn Thr Gly Lys Leu
Lys Asn Asp Lys Ile
<210> SEQ ID NO 81
<211> LENGTH: 69
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 81
Val Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Ala Pro
Leu Asp His Lys Asp Lys Gly Leu Lys Ser Leu Thr Leu Glu Asp Ser
Ile Ser Gln Asn Gly Thr Leu Thr Leu Ser Ala Gln Gly Ala Glu Arg
Thr Phe Lys Ala Gly Asn Lys Asp Asn Ser Leu Asn Thr Gly Lys Leu
Lys Asn Asp Lys Ile
<210> SEQ ID NO 82
<211> LENGTH: 69
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 82
Val Thr Ala Asp Ile Gly Thr Gly Leu Ala Asp Ala Leu Thr Ala Pro
                                      10
Leu Asp His Lys Asp Lys Gly Leu Lys Ser Leu Thr Leu Glu Asp Ser
           20
                                25
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Ile Pro Gln Asn Gly Thr Leu Thr Leu Ser Ala Gln Gly Ala Glu Lys
                          40
Thr Phe Lys Ala Gly Asp Lys Asp Asn Ser Leu Asn Thr Gly Lys Leu
Lys Asn Asp Lys Ile
<210> SEQ ID NO 83
<211> LENGTH: 69
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 83
Val Ala Ala Asp Ile Gly Ala Gly Leu Ala Asp Ala Leu Thr Ala Pro
Leu Asp His Lys Asp Lys Gly Leu Lys Ser Leu Thr Leu Glu Asp Ser
Ile Ser Gln Asn Gly Thr Leu Thr Leu Ser Ala Gln Gly Ala Glu Arg
Thr Phe Lys Ala Gly Asp Lys Asp Asn Ser Leu Asn Thr Gly Lys Leu
Lys Asn Asp Lys Val
<210> SEQ ID NO 84
<211> LENGTH: 69
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 84
Val Val Ala Asp Ile Gly Thr Gly Leu Ala Asp Ala Leu Thr Ala Pro
Leu Asp His Lys Asp Lys Gly Leu Lys Ser Leu Thr Leu Glu Asp Ser
Ile Pro Gln Asn Gly Thr Leu Thr Leu Ser Ala Gln Gly Ala Glu Lys
Thr Phe Lys Ala Gly Asp Lys Asp Asn Ser Leu Asn Thr Gly Lys Leu
Lys Asn Asp Lys Ile
<210> SEQ ID NO 85
<211> LENGTH: 15
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 85
Ile Arg Gln Ile Glu Val Asp Gly Gln Leu Ile Thr Leu Glu Ser
1 5
<210> SEQ ID NO 86
<211> LENGTH: 15
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
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<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 86
Ile Arg Gln Ile Glu Val Asp Gly Lys Leu Ile Thr Leu Glu Ser
<210> SEQ ID NO 87
<211> LENGTH: 15
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 87
Ile Arg Gln Ile Glu Val Asp Gly Gln Thr Ile Thr Leu Ala Ser
<210> SEQ ID NO 88
<211> LENGTH: 15
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 88
Ile Arg Gln Ile Glu Val Asp Arg Gln Leu Ile Thr Leu Glu Ser
<210> SEQ ID NO 89
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 89
Ile Arg Gln Ile Glu Val Asp Gly Gln Leu Ile Thr Leu Glu Asn
<210> SEQ ID NO 90
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 90
Ile His Gln Ile Glu Val Asp Gly Gln Leu Ile Thr Leu Glu Ser
<210> SEQ ID NO 91
<211> LENGTH: 15
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 91
Ile Arg Gln Ile Glu Val Asn Gly Gln Leu Ile Thr Leu Glu Ser
                                    10
<210> SEQ ID NO 92
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
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<400> SEQUENCE: 92
Ile Arg Gln Ile Arg Ser Asp Gly Gln Leu Ile Thr Leu Glu Ser
                                   10
<210> SEQ ID NO 93
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 93
Ile Arg Gln Ile Glu Val Asp Gly Gln Leu Ile Thr Leu Glu Arg
<210> SEQ ID NO 94
<211> LENGTH: 15
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 94
Ile Arg Gln Ile Glu Val Asp Gly Arg Leu Ile Thr Leu Glu Ser
                                    10
<210> SEQ ID NO 95
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 95
Val Gln Lys Ile Glu Val Asp Gly Gln Thr Ile Thr Leu Ala Ser
                                  10
1 5
<210> SEQ ID NO 96
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 96
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
Val Gln Asp Ser Glu Asp Ser Gly Lys Met Val Ala Lys Arg Gln Phe 20 \hspace{1cm} 25 \hspace{1cm} 30 \hspace{1cm}
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
Lys Gly Gly Ser Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser
   50
<210> SEQ ID NO 97
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 97
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
                          10
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Val Gln Asp Ser Glu His Ser Gly Lys Met Val Ala Lys Arg Gln Phe
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
Glu Gly Gly Arg Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser
<210> SEQ ID NO 98
<211> LENGTH: 62
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 98
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
Glu Gln Asp Pro Glu His Ser Gly Lys Met Val Ala Lys Arg Arg Phe
Lys Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
Lys Asp Val Met Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser
<210> SEQ ID NO 99
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 99
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
                         10
Val Gln Asp Ser Glu His Ser Gly Lys Met Val Ala Lys Arg Gln Phe
Arg Ile Gly Asp Ile Val Gly Glu His Thr Ser Phe Gly Lys Leu Pro
Lys Asp Val Met Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser
<210> SEQ ID NO 100
<211> LENGTH: 62
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 100
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Phe Gln Thr Glu Gln
                                  10
Ile Gln Asp Ser Glu His Ser Gly Lys Met Val Ala Lys Arg Gln Phe
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
                           40
Glu Gly Gly Arg Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser
<210> SEQ ID NO 101
<211> LENGTH: 62
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 101
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
Val Gln Asp Ser Glu Asp Ser Gly Lys Met Val Ala Lys Arg Gln Phe
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
Lys Asp Val Met Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser 50 60
<210> SEQ ID NO 102
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 102
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
Val Gln Asp Ser Glu His Ser Gly Ser Met Val Ala Lys Arg Gln Phe
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
Glu Gly Gly Arg Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser 50 55 60
<210> SEQ ID NO 103
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 103
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
Glu Gln Asp Pro Glu His Ser Glu Lys Met Val Ala Lys Arg Arg Phe
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
Lys Asp Val Met Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser
<210> SEQ ID NO 104
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 104
Val Tyr Lys Gln Ser Tyr Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
                        10
Glu Gln Asp Ser Glu His Ser Gly Lys Met Val Ala Lys Arg Arg Phe
                                25
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Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
      35
                             40
Lys Gly Ser Ser Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser
<210> SEQ ID NO 105
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 105
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
Glu Gln Asp Leu Glu His Ser Gly Lys Met Val Ala Lys Arg Arg Phe
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Arg
Glu Gly Gly Arg Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser 50 \\ 60
<210> SEQ ID NO 106
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 106
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
Val Gln Asp Ser Glu His Ser Gly Lys Met Val Ala Lys Arg Gln Phe
Arg Ile Gly Asp Ile Val Gly Glu His Thr Ser Phe Asp Lys Leu Pro
Lys Asp Val Met Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser
<210> SEQ ID NO 107
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 107
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Phe Gln Thr Glu Gln
Ile Gln Asp Ser Glu Asp Ser Gly Lys Met Val Ala Lys Arg Arg Phe 20 \hspace{1cm} 25 \hspace{1cm} 30 \hspace{1cm}
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
                             40
Lys Gly Gly Ser Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser
    50
<210> SEQ ID NO 108
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
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<400> SEQUENCE: 108
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
Val Gln Asp Ser Glu His Ser Gly Lys Met Val Ala Lys Arg Arg Phe
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
Glu Ser Asp Arg Ala Thr Tyr Arg Gly Thr Ala Phe Ser Ser
<210> SEQ ID NO 109
<211> LENGTH: 62
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 109
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
                                   10
Val Gln Asp Ser Glu His Ser Arg Lys Met Val Ala Lys Arg Gln Phe 20 \hspace{1cm} 25 \hspace{1cm} 30 \hspace{1cm}
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
                            40
Lys Gly Asp Ser Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser
<210> SEQ ID NO 110
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 110
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
1
                          10
Val Gln Asp Ser Glu Asp Ser Gly Lys Met Val Ala Lys Arg Gln Phe
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
Lys Gly Gly Ser Ala Thr Tyr Arg Gly Thr Ala Leu Gly Ser
<210> SEQ ID NO 111
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 111
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
                                   10
Val Gln Asp Ser Glu Asp Ser Gly Lys Met Val Ala Lys Arg Gln Phe
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
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Lys Gly Gly Ser Ala Thr Tyr Arg Gly Thr Ala Phe Ser Ser

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                                              60
<210> SEQ ID NO 112
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 112
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
Val Gln Asp Ser Glu His Ser Gly Lys Met Val Ala Lys Arg Gln Phe 20 \hspace{1cm} 25 \hspace{1cm} 30 \hspace{1cm}
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
Lys Gly Gly Ser Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser 50 \\
<210> SEQ ID NO 113
<211> LENGTH: 62
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 113
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
                                10
Val Gln Asp Ser Glu His Ser Arg Lys Met Val Ala Lys Arg Gln Phe
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
Glu Gly Gly Arg Ala Thr Tyr Arg Gly Thr Ala Leu Gly Ser
<210> SEQ ID NO 114
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 114
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
Val Gln Asp Ser Glu His Ser Gly Lys Met Val Ala Lys Arg Gln Phe
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
Glu Gly Gly Arg Ala Thr Tyr Arg Gly Thr Ala Phe Ser Ser
                        55
<210> SEQ ID NO 115
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 115
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Phe Gln Thr Glu Gln
```

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10
Ile Gln Asp Ser Glu His Ser Gly Lys Met Val Ala Lys Arg Arg Phe
                                  25
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
Glu Gly Gly Arg Ala Thr Tyr Arg Gly Thr Ala Phe Ser Ser
<210> SEQ ID NO 116
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 116
Val Tyr Lys Gln Ser Tyr Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
Ile Gln Asp Ser Glu His Ser Gly Lys Met Val Ala Lys Arg Arg Phe 20 \hspace{1cm} 25 \hspace{1cm} 30 \hspace{1cm}
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Phe Phe Asp Lys Leu Pro
Lys Gly Gly Ser Ala Ile Tyr Arg Gly Thr Ala Phe Gly Ser 50 \\
<210> SEO ID NO 117
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEOUENCE: 117
\label{thm:condition} \mbox{Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Phe Gln Thr Glu Gln}
                                      10
Ile Gln Asp Ser Glu His Ser Gly Lys Met Val Ala Lys Arg Gln Phe
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
Glu Gly Gly Arg Ala Thr Tyr Arg Gly Thr Ala Phe Ser Ser
<210> SEQ ID NO 118
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 118
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Phe Gln Thr Glu Gln
                                      10
Ile Gln Asp Ser Glu His Ser Gly Lys Met Val Ala Lys Arg Arg Phe
                                  25
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
                             40
Glu Gly Gly Arg Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser
    50
```

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<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 119
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
Ile Gln Asp Ser Glu His Ser Gly Lys Met Val Ala Lys Arg Gln Phe
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
Glu Gly Gly Arg Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser
<210> SEQ ID NO 120
<211> LENGTH: 62
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 120
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
Val Gln Asp Ser Glu His Ser Gly Lys Met Val Ala Lys Arg Gln Phe 20 \hspace{1cm} 25 \hspace{1cm} 30 \hspace{1cm}
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
                            40
Lys Gly Gly Arg Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser
<210> SEQ ID NO 121
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 121
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
Val Gln Asp Ser Glu His Ser Gly Lys Met Val Ala Lys Arg Gln Phe
Arg Ile Gly Asp Ile Ala Gly Glu His Ile Ser Phe Asp Lys Leu Pro
Glu Gly Gly Arg Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser 50 \\
<210> SEQ ID NO 122
<211> LENGTH: 62
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 122
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
                                     10
Val Gln Asp Ser Glu Asp Ser Gly Lys Met Val Ala Lys Arg Gln Phe
```

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Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
                             40
Glu Gly Gly Arg Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser
<210> SEQ ID NO 123
<211> LENGTH: 62
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 123
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Phe Gln Thr Glu Gln
Ile Gln Asp Ser Glu His Ser Gly Lys Met Val Ala Lys Arg Gln Phe 20 \hspace{1cm} 25 \hspace{1cm} 30 \hspace{1cm}
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
Lys Gly Gly Ser Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser
<210> SEQ ID NO 124
<211> LENGTH: 62
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 124
\label{thm:condition} \mbox{Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln}
1
                                      10
Val Gln Asp Ser Glu Asp Ser Gly Lys Met Val Ala Lys Arg Gln Phe
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
Lys Gly Asp Ser Ala Thr Tyr Arg Gly Thr Ala Phe Ser Ser
<210> SEQ ID NO 125
<211> LENGTH: 62
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 125
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
Val Gln Asp Ser Glu Asp Ser Gly Lys Met Val Ala Lys Arg Gln Phe
                                25
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
Glu Ser Asp Ser Ala Thr Tyr Arg Gly Thr Ala Phe Ser Ser
                        55
<210> SEQ ID NO 126
<211> LENGTH: 62
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
```

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<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 126
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
Val Gln Asp Ser Glu Asp Ser Gly Lys Met Val Ala Lys Arg Gln Phe
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
Lys Gly Gly Arg Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser
<210> SEQ ID NO 127
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 127
\label{thm:condition} \mbox{Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln}
Val Gln Asp Ser Glu Asp Ser Gly Lys Met Val Ala Lys Arg Gln Phe
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu His
Lys Gly Gly Ser Ala Thr Tyr Arg Gly Thr Ala Phe Ser Ser
<210> SEQ ID NO 128
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 128
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
Val Gln Asp Ser Glu Asp Ser Arg Lys Met Val Ala Lys Arg Gln Phe
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
Glu Ser Asp Arg Ala Thr Tyr Arg Gly Thr Ala Phe Ser Ser
<210> SEQ ID NO 129
<211> LENGTH: 62
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 129
\label{thm:conditional} \mbox{Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln}
Glu Gln Asp Pro Glu His Ser Gly Lys Met Val Ala Lys Arg Arg Phe
                      25
Lys Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
```

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Lys Asp Val Met Val Thr Tyr Arg Gly Thr Ala Phe Gly Ser
   50
<210> SEQ ID NO 130
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 130
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
Val Gln Asp Ser Glu His Ser Ala Lys Met Val Ala Lys Arg Gln Phe 20 \hspace{1cm} 25 \hspace{1cm} 30 \hspace{1cm}
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
Glu Gly Gly Arg Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser
<210> SEQ ID NO 131
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 131
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
Glu Gln Asp Pro Glu His Ser Glu Lys Met Val Ala Lys Arg Arg Phe
Lys Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
                            40
Lys Asp Val Met Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser
<210> SEQ ID NO 132
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 132
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
Val Gln Asp Ser Glu His Ser Glu Lys Met Val Ala Lys Arg Arg Phe
Lys Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
Lys Asp Val Met Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser
<210> SEQ ID NO 133
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 133
```

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Val Tyr Lys Gln Ser Tyr Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
                                      10
Val Gln Asp Ser Glu Asp Ser Gly Lys Met Val Ala Lys Arg Gln Phe
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
Lys Gly Gly Ser Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser
<210> SEQ ID NO 134
<211> LENGTH: 62
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 134
Val Tyr Lys Gln Ser Tyr Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
                               10
Val Gln Asp Ser Glu His Ser Gly Lys Met Val Ala Lys Arg Arg Phe 20 \hspace{1cm} 25 \hspace{1cm} 30 \hspace{1cm}
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
                             40
Gly Asp Ser Arg Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser
<210> SEQ ID NO 135
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 135
\label{thm:condition} \mbox{Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln}
Val Gln Asp Ser Glu His Ser Gly Lys Met Val Ala Lys Arg Arg Phe
                                  25
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
Glu Gly Gly Arg Ala Thr Tyr Arg Gly Thr Ala Phe Ser Ser
<210> SEQ ID NO 136
<211> LENGTH: 62
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 136
\label{thm:condition} \mbox{Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln}
                                      10
Val Gln Asp Ser Glu Asp Ser Gly Lys Met Val Ala Lys Arg Gln Phe
                                  25
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
Glu Asp Val Arg Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser
                         55
```

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<210> SEQ ID NO 137
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 137
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
Val Gln Asp Ser Glu His Ser Gly Lys Met Val Ala Lys Arg Gln Phe 20 \hspace{1cm} 25 \hspace{1cm} 30 \hspace{1cm}
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
Glu Gly Val Arg Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser
<210> SEQ ID NO 138
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEOUENCE: 138
\label{thm:condition} \mbox{Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Phe Gln Thr Glu Gln}
                                     10
Glu Gln Asp Ser Glu His Ser Gly Lys Met Val Ala Lys Arg Gln Phe
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
                             40
Asp Val Val Arg Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser
<210> SEQ ID NO 139
<211> LENGTH: 62
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 139
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
Val Gln Asp Ser Glu His Ser Gly Lys Met Val Ala Lys Arg Gln Phe
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
Glu Ser Gly Arg Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser
<210> SEQ ID NO 140
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 140
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
              5
Glu Gln Asp Leu Glu His Ser Gly Lys Met Val Ala Lys Arg Arg Phe
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30 Lys Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro 35 40 Lys Asp Val Met Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser <210> SEQ ID NO 141 <211> LENGTH: 62 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: fHbp sequence variant <400> SEQUENCE: 141 Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln Val Gln Asp Ser Glu His Ser Glu Lys Met Val Ala Lys Arg Gln Phe  $20 \hspace{1cm} 25 \hspace{1cm} 30 \hspace{1cm}$ Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro Glu Ser Gly Ser Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser 50  $\,$  55  $\,$  60 <210> SEQ ID NO 142 <211> LENGTH: 62 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: fHbp sequence variant <400> SEQUENCE: 142  $\label{thm:condition} \mbox{Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Phe Gln Thr Glu Gln}$ Val Gln Asp Ser Glu His Ser Gly Lys Met Val Ala Lys Arg Gln Phe Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro Glu Gly Gly Arg Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser <210> SEQ ID NO 143 <211> LENGTH: 62 <212> TYPE: PRT <213 > ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: fHbp sequence variant <400> SEQUENCE: 143 Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln 10 Val Gln Asp Ser Glu His Ser Gly Lys Met Val Ala Lys Arg Gln Phe 25 Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro 40 Lys Gly Val Arg Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser <210> SEQ ID NO 144 <211> LENGTH: 62 <212> TYPE: PRT <213 > ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 144
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
Glu Gln Asp Leu Glu His Ser Arg Lys Met Val Ala Lys Arg Arg Phe \phantom{-}20\phantom{+}25\phantom{+} 30
Lys Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
Lys Asp Val Met Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser
<210> SEQ ID NO 145
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 145
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
Glu Gln Asp Leu Glu His Ser Arg Lys Met Val Ala Lys Arg Arg Phe 20 \hspace{1cm} 25 \hspace{1cm} 30 \hspace{1cm}
Lys Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
                             40
Lys Asp Val Met Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser
<210> SEQ ID NO 146
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 146
\label{thm:condition} \mbox{Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln}
Glu Gln Asp Pro Glu His Ser Gly Lys Met Val Ala Lys Arg Arg Phe
Lys Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
Lys Asp Ile Met Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser
<210> SEQ ID NO 147
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 147
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
Val Gln Asp Ser Glu His Ser Gly Arg Met Val Ala Lys Arg Gln Phe
                                  25
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
                      40
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Glu Gly Gly Arg Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser
<210> SEQ ID NO 148
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 148
Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
Val Gln Asp Ser Glu His Phe Gly Lys Met Val Ala Lys Arg Gln Phe 20 \hspace{1cm} 25 \hspace{1cm} 30 \hspace{1cm}
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
Glu Gly Gly Arg Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser
<210> SEQ ID NO 149
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 149
\label{thm:condition} \mbox{Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln}
Val Gln Asp Ser Glu His Ser Gly Lys Met Val Ala Lys Arg Arg Phe
                                  25
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
Lys Asp Gly Met Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser
<210> SEQ ID NO 150
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 150
Ile Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Phe Gln Thr Glu Gln
Ile Gln Asp Ser Glu His Ser Gly Lys Met Val Ala Lys Arg Gln Phe \phantom{-}20\phantom{+}25\phantom{+} 30
Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
                             40
Glu Gly Gly Arg Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser
<210> SEQ ID NO 151
<211> LENGTH: 62
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 151
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Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Leu Gln Thr Glu Gln
Val Gln Asp Ser Glu Asp Ser Gly Lys Met Val Ala Lys Arg Arg Phe 20 \hspace{1cm} 25 \hspace{1cm} 30 \hspace{1cm}
Lys Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu Pro
Lys Asp Val Met Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser
<210> SEQ ID NO 152
<211> LENGTH: 61
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 152
Ile Tyr Lys Gln Asp His Ser Ala Val Val Ala Leu Gln Ile Glu Lys
Ile Asn Asn Pro Asp Lys Ile Asp Ser Leu Ile Asn Gln Arg Ser Phe
                               25
Leu Val Ser Gly Leu Gly Gly Glu His Thr Ala Phe Asn Gln Leu Pro
                            40
Gly Gly Lys Ala Glu Tyr His Gly Lys Ala Phe Ser Ser
                        55
<210> SEO ID NO 153
<211> LENGTH: 61
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 153
Ile Tyr Lys Gln Asp His Ser Ala Val Val Ala Leu Gln Ile Glu Lys
Ile Asn Asn Pro Asp Lys Ile Asp Ser Leu Ile Asn Gln Arg Ser Phe
Leu Val Ser Gly Leu Gly Gly Glu His Thr Ala Phe Asn Gln Leu Pro
                            40
Ser Gly Lys Ala Glu Tyr His Gly Lys Ala Phe Ser Ser
<210> SEQ ID NO 154
<211> LENGTH: 61
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 154
Ile Tyr Lys Gln Asn His Ser Ala Val Val Ala Leu Gln Ile Glu Lys
Ile Asn Asn Pro Asp Lys Ile Asp Ser Leu Ile Asn Gln Arg Ser Phe
                              25
Leu Val Ser Gly Leu Gly Gly Glu His Thr Ala Phe Asn Gln Leu Pro
                 40
Asp Gly Lys Ala Glu Tyr His Gly Lys Ala Phe Ser Ser
                        55
```

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<210> SEQ ID NO 155
<211> LENGTH: 61
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 155
Ile Tyr Lys Gln Asp His Ser Ala Val Val Ala Leu Gln Ile Glu Lys
Ile Asn Asn Pro Asp Lys Ile Asp Ser Leu Ile Asn Gln Arg Ser Phe
Leu Val Ser Gly Leu Gly Gly Glu His Thr Ala Phe Asn Gln Leu Pro
Asp Gly Lys Ala Glu Tyr His Gly Lys Ala Phe Ser Ser
<210> SEQ ID NO 156
<211> LENGTH: 61
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 156
Ile Tyr Lys Gln Asn His Ser Ala Val Val Ala Leu Gln Ile Glu Lys
Ile Asn Asn Pro Asp Lys Ile Asp Ser Leu Ile Asn Gln Arg Ser Phe
Leu Val Ser Gly Leu Gly Gly Glu His Thr Ala Phe Asn Gln Leu Pro
                           40
Gly Gly Lys Ala Glu Tyr His Gly Lys Ala Phe Ser Ser
                        55
<210> SEQ ID NO 157
<211> LENGTH: 61
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 157
Ile Tyr Lys Gln Asp His Ser Ala Val Val Ala Leu Gln Ile Glu Lys
Ile Asn Asn Pro Asp Lys Ile Asp Ser Leu Ile Asn Gln Arg Ser Phe 20 \hspace{1cm} 25 \hspace{1cm} 30 \hspace{1cm}
Leu Val Ser Gly Leu Gly Gly Glu His Thr Ala Phe Asn Gln Leu Pro
Ser Gly Lys Ala Glu Tyr His Gly Lys Ala Phe Ser Phe
<210> SEQ ID NO 158
<211> LENGTH: 61
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 158
Ile Tyr Lys Gln Asp His Ser Ala Val Val Ala Leu Gln Ile Glu Lys
```

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Ile Asn Asn Pro Asp Lys Ile Asp Ser Leu Ile Asn Arg Arg Ser Phe
Leu Val Ser Gly Leu Gly Gly Glu His Thr Ala Phe Asn Gln Leu Pro
Asp Gly Lys Ala Glu Tyr His Gly Lys Ala Phe Ser Ser
<210> SEQ ID NO 159
<211> LENGTH: 61
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 159
Ile Tyr Lys Gln Asn His Ser Ala Val Val Ala Leu Gln Ile Glu Lys
Ile Asn Asn Pro Asp Lys Ile Asp Ser Leu Ile Asn Gln Arg Ser Phe
Leu Val Ser Gly Leu Gly Gly Glu His Thr Ala Phe Asn Gln Leu Pro
                           40
Val Gly Lys Ala Glu Tyr His Gly Lys Ala Phe Ser Ser
                       55
<210> SEQ ID NO 160
<211> LENGTH: 61
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEOUENCE: 160
Ile Tyr Lys Gln Asn His Ser Ala Val Val Ala Leu Gln Ile Glu Lys
                                   10
Ile Asn Asn Pro Asp Lys Thr Asp Ser Leu Ile Asn Gln Arg Ser Phe
                               25
Leu Val Ser Gly Leu Gly Gly Glu His Thr Ala Phe Asn Gln Leu Pro
                            40
Gly Gly Lys Ala Glu Tyr His Gly Lys Ala Phe Ser Ser
<210> SEQ ID NO 161
<211> LENGTH: 61
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 161
Ile Tyr Lys Gln Asp His Ser Ala Val Val Ala Leu Gln Ile Glu Lys
Ile Asn Asn Pro Asp Lys Thr Asp Ser Leu Ile Asn Gln Arg Ser Phe
                               25
Leu Val Ser Gly Leu Gly Gly Glu His Thr Ala Phe Asn Gln Leu Pro
                           40
Gly Gly Lys Ala Glu Tyr His Gly Lys Ala Phe Ser Ser
<210> SEQ ID NO 162
<211> LENGTH: 61
<212> TYPE: PRT
```

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<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 162
Ile Tyr Lys Gln Asn His Ser Ala Val Val Ala Leu Gln Ile Glu Lys
                         10
Ile Asn Asn Pro Asp Lys Ile Asp Ser Leu Ile Asn Gln Arg Ser Phe
Leu Val Ser Gly Leu Gly Gly Glu His Thr Ala Phe Asn Gln Leu Pro
Gly Asp Lys Ala Glu Tyr His Gly Lys Ala Phe Ser Ser
<210> SEQ ID NO 163
<211> LENGTH: 61
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 163
Ile Tyr Lys Gln Asp His Ser Ala Val Val Ala Leu Gln Ile Glu Lys
Ile Asn Asn Pro Asp Lys Ile Asp Ser Leu Ile Asn Gln Arg Ser Phe
                               25
Leu Val Ser Gly Leu Gly Gly Glu His Thr Ala Phe Asn Gln Leu Pro
                          40
Val Asp Lys Ala Glu Tyr His Gly Lys Ala Phe Ser Ser
                       55
<210> SEQ ID NO 164
<211> LENGTH: 61
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 164
Ile Tyr Lys Gln Gly His Ser Ala Val Val Ala Leu Gln Ile Glu Lys
Ile Asn Asn Pro Asp Lys Ile Asp Ser Leu Ile Asn Gln Arg Ser Phe
Leu Val Ser Gly Leu Gly Gly Glu His Thr Ala Phe Asn Gln Leu Pro
Ser Gly Lys Ala Glu Tyr His Gly Lys Ala Phe Ser Ser
<210> SEQ ID NO 165
<211> LENGTH: 61
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 165
Ile Tyr Lys Gln Asp His Ser Ala Val Val Ala Leu Gln Ile Glu Lys
Ile Asn Asn Pro Asp Lys Ile Asp Ser Leu Ile Asn Gln Arg Ser Phe
                               25
Leu Val Ser Gly Leu Gly Gly Glu His Thr Ala Phe Asn Gln Leu Pro
```

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Ser Gly Lys Ala Glu Tyr His Gly Lys Ala Leu Ser Ser
                        55
<210> SEQ ID NO 166
<211> LENGTH: 61
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 166
Ile Tyr Lys Gln Asp His Ser Ala Val Val Ala Leu Gln Ile Glu Lys
Ile Asn Asn Pro Asp Lys Ile Asp Ser Leu Ile Asn Gln Arg Ser Phe
Leu Val Ser Gly Leu Gly Gly Glu His Thr Ala Phe Asn Gln Leu Pro
Gly Asp Lys Ala Glu Tyr His Gly Lys Ala Phe Ser Ser
<210> SEQ ID NO 167
<211> LENGTH: 61
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 167
Ile Tyr Lys Gln Asp His Ser Ala Val Val Ala Leu Gln Thr Glu Lys
Val Asn Asn Pro Asp Lys Thr Asp Ser Leu Ile Asn Gln Arg Ser Phe
Leu Val Ser Gly Leu Gly Gly Glu His Thr Ala Phe Asn Gln Leu Pro
                            40
Val Gly Lys Ser Glu Tyr His Gly Lys Ala Phe Ser Ser
<210> SEQ ID NO 168
<211> LENGTH: 61
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 168
Ile Tyr Lys Gln Asn His Ser Ala Val Val Ala Leu Gln Ile Glu Lys
Ile Asn Asn Pro Asp Lys Ile Asp Ser Leu Ile Asn Gln Arg Ser Phe
Leu Val Ser Gly Leu Gly Gly Glu His Thr Ala Phe Asn Gln Leu Pro
                           40
Ser Gly Lys Ala Glu Tyr His Gly Lys Ala Phe Ser Ser
<210> SEQ ID NO 169
<211> LENGTH: 61
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
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<400> SEQUENCE: 169
Ile Tyr Lys Gln Asn His Ser Ala Val Val Ala Leu Gln Ile Glu Lys
1
                                   10
Ile Asn Asn Pro Asp Lys Ile Asp Ser Leu Ile Asn Gln Arg Ser Phe
                              25
Leu Val Ser Ser Leu Gly Gly Glu His Thr Ala Phe Asn Gln Leu Pro
Gly Gly Lys Ala Glu Tyr His Gly Lys Ala Phe Ser Ser
<210> SEQ ID NO 170
<211> LENGTH: 61
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 170
Val Tyr Lys Gln Asp His Ser Ala Val Val Ala Leu Gln Ile Glu Lys
Ile Asn Asn Pro Asp Lys Ile Asp Ser Leu Ile Asn Gln Arg Ser Phe
Leu Val Ser Gly Leu Gly Gly Glu His Thr Ala Phe Asn Gln Leu Pro
                         40
Gly Gly Lys Ala Glu Tyr His Gly Lys Ala Phe Ser Ser
   50
                       55
<210> SEQ ID NO 171
<211> LENGTH: 61
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 171
Ile Tyr Lys Gln Asp His Ser Ala Val Val Ala Leu Gln Thr Glu Lys
                                 10
Val Asn Asn Pro Asp Lys Thr Asp Ser Leu Ile Asn Gln Arg Ser Phe
                               25
Leu Val Ser Gly Leu Gly Gly Glu His Thr Ala Phe Asn Gln Leu Pro
Gly Gly Lys Ala Glu Tyr His Gly Lys Ala Phe Ser Ser
<210> SEQ ID NO 172
<211> LENGTH: 61
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 172
Ile Tyr Lys Gln Asp His Ser Ala Val Val Ala Leu Gln Ile Glu Lys
Ile Asn Asn Pro Asp Lys Ile Asp Ser Leu Ile Asn Gln Arg Ser Phe
                               25
Leu Val Ser Gly Leu Gly Gly Glu His Thr Ala Phe Asn Gln Leu Ser
                           40
Gly Gly Lys Ala Glu Tyr His Gly Lys Ala Phe Ser Ser
                      55
```

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<210> SEQ ID NO 173
<211> LENGTH: 61
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 173
Ile Tyr Lys Gln Asp His Ser Ala Val Val Ala Leu Gln Ile Glu Lys
Ile Asn Asn Pro Asp Lys Ile Asp Ser Leu Ile Asn Gln Arg Ser Phe
Leu Val Ser Gly Leu Gly Gly Lys His Thr Ala Phe Asn Gln Leu Pro
Gly Gly Lys Ala Glu Tyr His Gly Lys Ala Phe Ser Ser
<210> SEQ ID NO 174
<211> LENGTH: 19
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEOUENCE: 174
Ala Gly Gly Lys Leu Thr Tyr Thr Ile Asp Phe Ala Ala Lys Gln Gly
                                    1.0
His Gly Lys
<210> SEQ ID NO 175
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 175
Ala Gly Gly Lys Leu Ile Tyr Thr Ile Asp Phe Ala Ala Lys Gln Gly
His Gly Lys
<210> SEQ ID NO 176
<211> LENGTH: 19
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 176
Ala Ser Gly Lys Leu Thr Tyr Thr Ile Asp Phe Ala Ala Lys Gln Gly
His Gly Lys
<210> SEQ ID NO 177
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 177
```

Ala Gly Gly Lys Leu Thr Tyr Thr Ile Asp Phe Ala Ala Lys Gln Gly

```
10
                                                         15
Tyr Gly Lys
<210> SEQ ID NO 178
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 178
Ala Gly Gly Lys Leu Thr Tyr Thr Ile Asp Phe Ala Ala Lys Gln Gly
Asn Gly Lys
<210> SEQ ID NO 179
<211> LENGTH: 19
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 179
Ala Gly Gly Lys Leu Thr Tyr Thr Ile Asp Phe Ala Val Lys Gln Gly
                                   10
His Gly Lys
<210> SEQ ID NO 180
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 180
Ala Gly Gly Lys Leu Thr Tyr Thr Ile Asp Phe Ala Thr Lys Gln Gly
               5
                                    10
His Gly Lys
<210> SEQ ID NO 181
<211> LENGTH: 19
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 181
Ala Gly Gly Lys Leu Thr Tyr Thr Ile Asp Phe Ala Ser Lys Gln Gly
His Gly Lys
<210> SEQ ID NO 182
<211> LENGTH: 19
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 182
Ala Gly Gly Lys Leu Ile Tyr Thr Ile Asp Phe Ala Ala Lys Gln Gly
1
                                    10
Tyr Gly Lys
```

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<210> SEQ ID NO 183
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 183
Ala Arg Gly Lys Leu Thr Tyr Thr Ile Asp Phe Ala Ala Lys Gln Gly
                                    10
His Gly Lys
<210> SEQ ID NO 184
<211> LENGTH: 19
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 184
Ala Ser Gly Glu Leu Thr Tyr Thr Ile Asp Phe Ala Ala Lys Gln Gly
His Gly Lys
<210> SEQ ID NO 185
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 185
Thr Gly Gly Lys Leu Thr Tyr Thr Ile Asp Phe Ala Ala Lys Gln Gly
His Gly Lys
<210> SEQ ID NO 186
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 186
Ala Gly Gly Lys Leu Thr Tyr Thr Ile Asp Phe Val Ala Lys Gln Gly
                                    10
His Gly Lys
<210> SEQ ID NO 187
<211> LENGTH: 19
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 187
Ala Gly Gly Lys Leu Thr Tyr Thr Ile Asp Phe Ala Ala Lys Gln Gly
             5
                                   10
His Gly Arg
<210> SEQ ID NO 188
<211> LENGTH: 19
<212> TYPE: PRT
```

```
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 188
Ala Gly Gly Arg Leu Thr Tyr Thr Ile Asp Phe Ala Ala Lys Gln Gly
                                    10
Cys Gly Lys
<210> SEQ ID NO 189
<211> LENGTH: 19
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 189
Ala Gly Glu Lys Leu Thr Tyr Thr Ile Asp Phe Ala Ala Lys Gln Gly
His Gly Lys
<210> SEQ ID NO 190
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 190
Thr Gly Gly Lys Leu Ile Tyr Thr Ile Asp Phe Ala Ala Lys Gln Gly
His Gly Lys
<210> SEQ ID NO 191
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 191
Ala Gly Gly Lys Leu Thr Tyr Ile Ile Asp Phe Ala Ala Lys Gln Gly
Tyr Gly Lys
<210> SEQ ID NO 192
<211> LENGTH: 19
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 192
Pro Asn Gly Arg Leu His Tyr Ser Ile Asp Phe Thr Lys Lys Gln Gly
               5
                                   10
Tyr Gly Arg
<210> SEQ ID NO 193
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
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<400> SEQUENCE: 193
Pro Asn Gly Arg Leu His Tyr Thr Ile Asp Phe Thr Asn Lys Gln Gly
                                    10
Tyr Gly Arg
<210> SEQ ID NO 194
<211> LENGTH: 19
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 194
Pro Asn Gly Arg Leu His Tyr Ser Ile Asp Phe Thr Lys Lys Gln Gly
Tyr Gly Gly
<210> SEQ ID NO 195
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 195
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Tyr Ile Lys Pro Asp
Glu Lys His His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Ala
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Lys Ala Gln Glu
Val Ala Gly Ser Ala Glu Val Lys Thr Val Asn Gly Ile Arg His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 196
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 196
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ser Asp Ile Lys Pro Asp
Lys Lys Arg His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Ala
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile Arg His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 197
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
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<400> SEQUENCE: 197
Ser Pro Glu Leu Asn Val Glu Leu Ala Thr Ala Tyr Ile Lys Pro Asp
Glu Lys His His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
                                25
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile His His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 198
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 198
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Asp Ile Lys Pro Asp
Glu Lys His His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Ala
                                25
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
                           40
Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile Arg His Ile
                       55
Gly Leu Ala Ala
<210> SEQ ID NO 199
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 199
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Asp Ile Lys Pro Asp
Glu Lys Arg His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Ala
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Lys Ala Gln Glu
Val Ala Gly Ser Ala Glu Val Lys Thr Val Asn Gly Ile Arg His Ile
Gly Leu Ala Ala
65
<210> SEQ ID NO 200
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 200
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Ser Pro Glu Leu Asn Val Glu Leu Ala Thr Ala Tyr Ile Lys Pro Asp

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10
Glu Lys Arg His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
                                25
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile His His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 201
<211> LENGTH: 68
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 201
Ser Pro Glu Leu Asn Val Asp Leu Val Ser Ala Asp Ile Lys Pro Asp
Glu Lys Arg His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Lys Ala Gln Glu
                           40
Val Ala Gly Ser Ala Glu Val Lys Thr Val Asn Gly Ile Arg His Ile
   50
                      55
Gly Leu Ala Ala
65
<210> SEQ ID NO 202
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 202
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Asp Ile Lys Pro Asp
Gly Lys Arg His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Ala
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Lys Ala Gln Glu
Val Ala Gly Ser Ala Glu Val Lys Thr Val Asn Gly Ile Arg His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 203
<211> LENGTH: 68
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 203
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Asp Ile Lys Pro Asp
                                    10
Gly Lys Arg His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Ala
          20
                             25
```

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Glu Lys Ser Ser Tyr Ser Leu Gly Ile Phe Gly Gly Lys Ala Gln Glu
                          40
Val Ala Gly Ser Ala Glu Val Lys Thr Val Asn Gly Ile Arg His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 204
<211> LENGTH: 68
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 204
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Asp Ile Lys Pro Asp
Glu Lys His His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Ala
                              25
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Lys Ala Gln Glu
                          40
Val Ala Gly Ser Ala Glu Val Lys Thr Val Asn Gly Ile Arg His Ile
                      55
Gly Leu Ala Ala
<210> SEQ ID NO 205
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 205
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Asp Ile Lys Pro Asp
Glu Lys His His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Lys Ala Gln Glu
                 40
Val Ala Gly Ser Ala Glu Val Lys Thr Val Asn Gly Ile Arg His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 206
<211> LENGTH: 68
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 206
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Asp Ile Lys Pro Asp
      5
                                 10
Glu Lys His His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
                        25
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Glu Ala Gln Glu
                           40
```

```
Val Ala Gly Ser Ala Glu Val Lys Thr Ala Asn Gly Ile His His Ile
                                            60
Gly Leu Ala Ala
<210> SEQ ID NO 207
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 207
Ser Pro Glu Leu Asn Val Asp Leu Ala Val Ala Tyr Ile Lys Pro Asp
Glu Lys His His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Glu Lys Ala Gln Glu
                 40
Val Ala Gly Ser Ala Glu Val Lys Thr Ala Asn Gly Ile His His Ile
                      55
Gly Leu Ala Ala
65
<210> SEQ ID NO 208
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 208
Ser Pro Glu Leu Asn Val Asp Leu Ala Val Ala Tyr Ile Lys Pro Asp
                                   10
Glu Lys His His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
                              25
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Glu Lys Ala Gln Glu
                           40
Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile His His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 209
<211> LENGTH: 68
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 209
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Asp Ile Lys Pro Asp
Glu Lys His His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile His His Ile
Gly Leu Ala Ala
```

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65
<210> SEQ ID NO 210
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 210
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Tyr Ile Lys Pro Asp
Lys Lys Arg His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile His His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 211
<211> LENGTH: 68
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 211
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Tyr Ile Lys Pro Asp
                                   10
Glu Lys His His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
                           40
Val Ala Gly Ser Ala Glu Val Lys Thr Ala Asn Gly Ile Arg His Ile
Gly Leu Ala Ala
65
<210> SEQ ID NO 212
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 212
Ser Pro Glu Leu Asn Val Glu Leu Ala Ala Ala Tyr Ile Lys Pro Asp
Glu Lys His His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
                              25
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
                40
Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile Gln His Ile
Gly Leu Ala Ala
65
```

<210> SEQ ID NO 213

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<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 213
Ser Pro Glu Leu Asn Val Glu Leu Ala Thr Ala Asp Ile Lys Pro Asp
Glu Lys His His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile His His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 214
<211> LENGTH: 68
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEOUENCE: 214
Ser Pro Glu Leu Asn Val Glu Leu Ala Thr Ala Tyr Ile Lys Pro Asp
                                   10
Glu Lys His His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
                               25
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Lys Ala Gln Glu
                           40
Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile His His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 215
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 215
Ser Pro Glu Leu Asn Val Glu Leu Ala Thr Ala Tyr Ile Lys Pro Asp
Glu Lys Arg His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
                           40
Val Ala Gly Ser Ala Glu Val Lys Thr Val Asn Gly Ile Arg His Ile
Gly Leu Ala Ala
65
<210> SEQ ID NO 216
<211> LENGTH: 68
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
```

```
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 216
Ser Pro Glu Leu Asn Val Glu Leu Ala Thr Ala Tyr Ile Lys Pro Asp
Glu Lys Arg His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile Arg His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 217
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 217
Ser Pro Glu Leu Asn Val Glu Leu Ala Thr Ala Tyr Ile Lys Pro Asp
Glu Lys Arg His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile Gln His Ile
Gly Leu Ala Ala
65
<210> SEQ ID NO 218
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 218
Ser Pro Glu Leu Asn Val Glu Leu Ala Thr Ala Tyr Ile Lys Pro Asp
Glu Lys Arg His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
Val Ala Gly Ser Ala Glu Val Glu Thr Val Asn Gly Ile His His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 219
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 219
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```
Ser Pro Glu Leu Asn Val Asn Leu Ala Ala Ala Asp Ile Lys Pro Asp
                                   10
Glu Lys Arg His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
                               25
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Glu Lys Phe Gln Glu
                40
Val Ala Gly Ser Ala Glu Val Lys Thr Ala Asn Gly Ile His His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 220
<211> LENGTH: 68
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 220
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Tyr Ile Lys Pro Asp
                                   10
Glu Lys His Tyr Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
                               25
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Lys Ala Gln Glu
                           40
Val Ala Gly Ser Ala Glu Val Lys Thr Val Asn Gly Ile Arg His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 221
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 221
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Tyr Ile Lys Pro Asp
Glu Lys Arg His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Lys Ala Gln Glu
Val Ala Gly Ser Ala Glu Val Lys Thr Ala Asn Gly Ile His His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 222
<211> LENGTH: 68
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 222
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ser Asp Ile Lys Pro Asp
             5
Lys Lys Arg His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Ala
```

-continued

30 Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu 35 40 Val Ala Trp Gln Arg Arg Val Glu Thr Ala Asn Gly Ile Arg His Ile Gly Leu Ala Ala <210> SEQ ID NO 223 <211> LENGTH: 68 <212> TYPE: PRT <213 > ORGANISM: Artificial Sequence <223> OTHER INFORMATION: fHbp sequence variant <400> SEQUENCE: 223 Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Asp Ile Lys Pro Asp Glu Lys His His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp  $20 \hspace{1cm} 25 \hspace{1cm} 30 \hspace{1cm}$ Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Lys Ala Gln Glu Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile His His Ile 55 Gly Leu Ala Ala 65 <210> SEQ ID NO 224 <211> LENGTH: 68 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: fHbp sequence variant <400> SEQUENCE: 224 Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ser Asp Ile Lys Pro Asp Lys Lys Arg His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Ala 25 Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Arg Gln Ala Gln Glu Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile Arg His Ile Gly Leu Ala Ala <210> SEQ ID NO 225 <211> LENGTH: 68 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: fHbp sequence variant <400> SEQUENCE: 225 Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ser Asp Ile Lys Pro Asp Lys Lys Arg His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Ala 25 Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Lys Ala Gln Glu 40

```
Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile Arg His Ile
                       55
Gly Leu Ala Ala
65
<210> SEQ ID NO 226
<211> LENGTH: 68
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 226
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Asp Ile Lys Pro Asp
Lys Lys Arg His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Ala
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
                          40
Val Ala Gly Ser Ala Glu Val Glu Thr Val Asn Gly Ile Arg His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 227
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 227
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Asp Ile Lys Pro Asp
                             10
Glu Lys Arg His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
                               25
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile Gln His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 228
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 228
Ser Pro Glu Leu Asn Val Glu Leu Ala Ala Ala Tyr Ile Lys Pro Asp
Glu Lys Arg His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
                              25
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
                40
Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile Gln His Ile
                       55
```

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Gly Leu Ala Ala
65
<210> SEQ ID NO 229
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 229
Ser Pro Glu Leu Asn Val Glu Leu Ala Thr Ala Tyr Ile Lys Pro Asp
Glu Lys His His Ala Val Ile Ser Gly Phe Val Leu Tyr Asn Gln Asp
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile His His Ile
Gly Leu Ala Ala
65
<210> SEQ ID NO 230
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 230
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Tyr Ile Lys Pro Asp
Glu Lys Arg His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Lys Ala Gln Glu
Val Ala Gly Ser Ala Glu Val Lys Thr Val Asn Gly Ile Arg His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 231
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 231
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ser Asp Ile Lys Pro Asp 1 \phantom{\bigg|} 5 \phantom{\bigg|} 10 \phantom{\bigg|} 15
Lys Lys Arg His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn His Ala
                                 25
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
                            40
Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile Arg His Ile
Gly Leu Ala Ala
65
```

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<210> SEQ ID NO 232
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 232
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Tyr Ile Lys Pro Asp
Glu Lys Arg His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile Gln His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 233
<211> LENGTH: 68
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 233
Ser Pro Glu Leu Asn Val Asp Leu Val Ala Ala Asp Ile Lys Pro Asp
                                  10
Lys Lys Arg His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Ala
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile Arg His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 234
<211> LENGTH: 68
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 234
Ser Pro Glu Leu Asn Val Glu Leu Ala Thr Ala Tyr Ile Lys Pro Asp
Glu Lys Arg His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Ser Gly Gln Ala Gln Glu
                           40
Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile His His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 235
<211> LENGTH: 68
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
```

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<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 235
Ser Pro Glu Leu Asn Val Glu Leu Ala Thr Ala Tyr Ile Lys Pro Asp
Glu Lys Arg Tyr Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile His His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 236
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 236
Ser Pro Glu Leu Asn Val Asp Leu Ala Val Ala Tyr Ile Lys Pro Asp
Glu Lys His His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
                           40
Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile His His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 237
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 237
Ser Pro Glu Leu Asn Val Glu Leu Ala Thr Ala Tyr Ile Lys Pro Asp
Glu Lys His His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
Ile Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile His His Ile
   50
Gly Leu Ala Ala
<210> SEQ ID NO 238
<211> LENGTH: 68
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 238
```

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Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Asn Ile Glu Gln Asp
Glu Lys His His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Glu Lys Ala Gln Glu
Val Ala Gly Ser Ala Glu Val Lys Thr Ala Asn Gly Ile Arg His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 239
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 239
Ser Pro Glu Leu Asn Val Glu Leu Ala Thr Ala Tyr Ile Lys Pro Asp
Glu Lys Arg His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asn
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile His His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 240
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 240
Ser Pro Glu Leu Asn Val Glu Leu Ala Ala Ala Tyr Ile Lys Pro Asp
Glu Lys Arg His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile His His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 241
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 241
Ser Pro Glu Leu Asn Val Asp Leu Ala Val Ala Tyr Ile Lys Pro Asp
                                    10
```

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Glu Lys His His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
                               25
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Glu Lys Ala Gln Glu
Val Ala Gly Ser Ala Glu Val Lys Thr Val Asn Gly Ile Arg His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 242
<211> LENGTH: 68
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 242
Ser Pro Glu Leu Asn Val Asp Leu Ala Val Ala Tyr Ile Lys Pro Asp
Glu Lys His His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
                              25
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Lys Ala Gln Glu
                           40
Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile His His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 243
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 243
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Tyr Ile Lys Pro Asp
1
                                   10
Glu Lys His His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile His His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 244
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 244
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Asp Ile Lys Pro Asp
Glu Lys His His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Ala
                              25
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
```

```
Val Ala Gly Ser Ala Glu Val Lys Thr Val Asn Gly Ile Arg His Ile
                         55
Gly Leu Ala Ala
<210> SEQ ID NO 245
<211> LENGTH: 68
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 245
Ser Pro Glu Leu Asn Val Asn Leu Ala Ala Ala Asp Ile Lys Pro Asp
Glu Lys Arg His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Ala 20 \\ 25 \\ 30
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Glu Lys Ala Gln Glu
Val Ala Gly Ser Ala Glu Val Lys Thr Ala Asn Gly Ile His His Ile 50 \phantom{000}55\phantom{000} 60
Gly Leu Ala Ala
65
<210> SEO ID NO 246
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 246
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Tyr Ile Lys Pro Asp
Glu Lys His His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Lys Ala Gln Glu
Val Ala Gly Ser Ala Glu Val Glu Thr Val Asn Gly Ile Arg His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 247
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 247
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Asp Ile Lys Pro Asp
                            10
Glu Lys Arg His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
                                 25
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Lys Ala Gln Glu
                            40
Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile Gln His Ile
                     55
```

```
Gly Leu Ala Ala
<210> SEQ ID NO 248
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 248
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Asp Ile Lys Pro Asp
Glu Lys His His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Glu Lys Ala Gln Glu
Val Ala Gly Ser Ala Glu Val Lys Thr Ala Asn Gly Ile His His Ile
Gly Leu Ala Ala
65
<210> SEQ ID NO 249
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 249
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Tyr Ile Lys Pro Asp
                                   10
Glu Lys His His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
                              25
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Lys Ala Gln Glu
Val Ala Gly Ser Ala Glu Val Lys Thr Val Asn Gly Ile Arg His Ile
Gly Leu Ala Ala
65
<210> SEQ ID NO 250
<211> LENGTH: 68
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 250
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Asp Ile Lys Pro Asp
                                   10
Glu Lys Arg His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
                           40
Val Ala Gly Ser Ala Glu Val Lys Thr Val Asn Gly Ile Arg His Ile
Gly Leu Ala Ala
```

```
<210> SEQ ID NO 251
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 251
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Tyr Ile Lys Pro Asp
Glu Lys His His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile Gln His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 252
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 252
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Tyr Ile Lys Pro Asp
Lys Lys Arg His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Lys Ala Gln Glu
                           40
Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile His His Ile
Gly Leu Ala Ala
65
<210> SEQ ID NO 253
<211> LENGTH: 68
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 253
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Tyr Ile Lys Pro Asp
Glu Lys His His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Ala
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
                           40
Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile Arg His Ile
                       55
   50
Gly Leu Ala Ala
<210> SEQ ID NO 254
<211> LENGTH: 68
<212> TYPE: PRT
```

```
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 254
Ser Pro Glu Leu Asn Val Glu Leu Ala Thr Ala Tyr Ile Lys Pro Asp
Glu Lys Arg His Ala Val Ile Ser Asp Ser Val Leu Tyr Asn Gln Asp
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile His His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 255
<211> LENGTH: 68
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEOUENCE: 255
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Tyr Ile Lys Pro Asp
                                   10
Glu Lys His His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Lys Ala Glu Glu
                           40
Val Ala Gly Ser Ala Glu Val Lys Thr Val Asn Gly Ile Arg His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 256
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 256
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Asn Ile Glu Gln Asp
Glu Lys His His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Ala
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Glu Lys Phe Gln Glu
Val Ala Gly Ser Ala Glu Val Lys Thr Val Asn Gly Ile Arg His Ile
                       55
Gly Leu Ala Ala
<210> SEQ ID NO 257
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
```

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<400> SEQUENCE: 257
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Tyr Ile Lys Pro Asp
                                   10
Glu Lys His His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Ala
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Lys Ala Gln Glu
Val Ala Gly Ser Ala Glu Val Lys Thr Val Asn Gly Ile Leu His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 258
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 258
Ser Pro Glu Leu Asn Val Glu Leu Ala Thr Ala Tyr Ile Lys Pro Asp
Glu Lys His His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
                              25
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
                          40
Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile Arg His Ile
Gly Leu Ala Ala
65
<210> SEQ ID NO 259
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 259
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Asp Ile Lys Pro Asp
Glu Lys His His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
                               25
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
Val Ala Gly Ser Ala Glu Val Lys Thr Val Asn Gly Ile Arg His Ile
Gly Leu Ala Ala
65
<210> SEQ ID NO 260
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 260
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ser Asp Ile Lys Pro Asp
1 5
                             10
```

```
Lys Lys Arg His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Ala
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
                           40
Val Ala Gly Ser Ala Glu Val Lys Thr Val Asn Gly Ile Arg His Ile
Gly Leu Ala Ala
65
<210> SEQ ID NO 261
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 261
Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Asp Ile Lys Pro Asp
Glu Lys Arg His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Asp
                               25
Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Gln Ala Gln Glu
                          40
Val Ala Gly Ser Ala Glu Val Glu Thr Ala Asn Gly Ile His His Ile
Gly Leu Ala Ala
<210> SEQ ID NO 262
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 262
Thr Pro Glu Gln Asn Val Glu Leu Ala Ser Ala Glu Leu Lys Ala Asp
Glu Lys Ser His Ala Val Ile Leu Gly Asp Thr Arg Tyr Gly Glu
                              25
Glu Lys Gly Thr Tyr His Leu Ala Leu Phe Gly Asp Arg Ala Gln Glu
Ile Ala Gly Ser Ala Thr Val Lys Ile Arg Glu Lys Val His Glu Ile
Gly Ile Ala Gly
<210> SEQ ID NO 263
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 263
Thr Pro Glu Gln Asn Val Glu Leu Ala Ala Ala Glu Leu Lys Ala Asp
                        10
Glu Lys Ser His Ala Val Ile Leu Gly Asp Thr Arg Tyr Gly Ser Glu
                               25
```

```
Glu Lys Gly Thr Tyr His Leu Ala Leu Phe Gly Asp Arg Ala Gln Glu
       35
Ile Ala Gly Ser Ala Thr Val Lys Ile Gly Glu Lys Val His Glu Ile
                      55
Gly Ile Ala Gly
65
<210> SEQ ID NO 264
<211> LENGTH: 68
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 264
Thr Pro Glu Gln Asn Val Glu Leu Ala Ser Ala Glu Leu Lys Ala Asp
Glu Lys Ser His Ala Val Ile Leu Gly Asp Thr Arg Tyr Gly Ser Glu
                    25
Glu Lys Gly Thr Tyr His Leu Ala Leu Phe Gly Asp Arg Ala Gln Glu
                          40
Ile Ala Gly Ser Ala Thr Val Lys Ile Arg Glu Lys Val His Glu Ile
   50
                       55
Gly Ile Ala Gly
65
<210> SEQ ID NO 265
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 265
Thr Pro Glu Gln Asn Val Glu Leu Ala Ser Ala Glu Leu Lys Ala Asp
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Glu Lys Ser His Ala Val Ile Leu Gly Asp Thr Arg Tyr Gly Ser Glu
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Glu Lys Gly Thr Tyr His Leu Ala Leu Phe Gly Asp Arg Ala Gln Glu
Ile Ala Gly Ser Ala Thr Val Lys Ile Gly Glu Lys Val His Glu Ile
Gly Ile Ala Gly
<210> SEQ ID NO 266
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
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Thr Pro Glu Leu Asn Val Asp Leu Ala Ser Ala Glu Leu Lys Ala Asp
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Glu Lys Ser His Ala Val Ile Leu Gly Asp Thr Arg Tyr Gly Ser Glu
Glu Lys Gly Thr Tyr His Leu Ala Leu Phe Gly Asp Arg Ala Gln Glu
Ile Ala Gly Ser Ala Thr Val Lys Ile Gly Glu Lys Val His Glu Ile
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Gly Ile Ala Gly
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<210> SEQ ID NO 267
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 267
Thr Leu Glu Gln Asn Val Glu Leu Ala Ala Glu Leu Lys Ala Asp
Glu Lys Ser His Ala Val Ile Leu Gly Asp Thr Arg Tyr Gly Ser Glu
Glu Lys Gly Thr Tyr His Leu Ala Leu Phe Gly Asp Arg Ala Gln Glu
Ile Ala Gly Ser Ala Thr Val Lys Ile Gly Glu Lys Val His Glu Ile
Gly Ile Ala Gly
<210> SEQ ID NO 268
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 268
Thr Pro Glu Gln Asn Val Glu Leu Ala Ala Glu Leu Lys Ala Asp
Glu Lys Ser His Ala Val Ile Leu Gly Asp Thr Arg Tyr Gly Glu
                               25
Glu Lys Gly Thr Tyr His Leu Ala Leu Phe Gly Asp Arg Ala Gln Glu
Ile Ala Gly Ser Ala Thr Val Lys Ile Arg Glu Lys Val His Glu Ile
Gly Ile Ala Gly
<210> SEQ ID NO 269
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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Thr Pro Glu Gln Asn Val Glu Leu Ala Ala Ala Glu Leu Lys Ala Asp
                                   10
Glu Lys Ser His Ala Val Ile Leu Gly Asp Thr Arg Tyr Asp Ser Glu
                              25
Glu Lys Gly Thr Tyr His Leu Ala Leu Phe Gly Asp Arg Ala Gln Glu
                           40
Ile Ala Gly Ser Ala Thr Val Lys Ile Gly Glu Lys Val His Glu Ile
   50
                       55
Ser Ile Ala Gly
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-continued

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<210> SEQ ID NO 270
<211> LENGTH: 68
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 270
Thr Pro Glu Gln Asn Val Glu Leu Ala Ala Glu Leu Lys Ala Asp
Glu Lys Ser His Ala Val Ile Leu Gly Asp Thr Arg Tyr Gly Ser Glu
Glu Lys Gly Thr Tyr His Leu Ala Leu Phe Gly Asp Arg Ala Gln Glu
Ile Ala Gly Ser Ala Thr Val Lys Ile Gly Glu Lys Val His Glu Ile
Ser Ile Ala Gly
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<210> SEQ ID NO 271
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 271
Ser Pro Glu Leu Asn Val Glu Leu Ala Thr Ala Glu Leu Lys Ala Asp
                                   10
Glu Lys Ser His Ala Val Ile Leu Gly Asp Thr Arg Tyr Gly Ser Glu
                               25
Glu Lys Gly Thr Tyr His Leu Ala Leu Phe Gly Asp Arg Ala Gln Glu
                          40
Ile Ala Gly Ser Ala Thr Val Lys Ile Arg Glu Lys Val His Glu Ile
Gly Ile Ala Gly
<210> SEQ ID NO 272
<211> LENGTH: 68
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
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Thr Pro Glu Gln Asn Val Glu Leu Ala Ser Ala Glu Leu Lys Ala Asp
Glu Lys Ser His Ala Val Ile Leu Gly Asp Thr Arg Tyr Gly Glu
                              25
Glu Lys Gly Thr Tyr His Leu Ala Leu Phe Gly Asp Arg Ala Gln Glu
Ile Ala Gly Ser Ala Thr Val Lys Ile Arg Glu Lys Val His Glu Ile
                       55
Asp Ile Ala Gly
<210> SEQ ID NO 273
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<211> LENGTH: 68

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<212> TYPE: PRT
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Thr Pro Glu Gln Asn Val Glu Leu Ala Ala Glu Leu Lys Ala Asp
Glu Lys Ser His Ala Val Ile Leu Gly Asp Thr His Tyr Gly Ser Glu
                               25
Glu Lys Gly Thr Tyr His Leu Ala Leu Phe Gly Asp Arg Ala Gln Glu
Ile Ala Gly Ser Ala Thr Val Lys Ile Gly Glu Lys Val His Glu Ile
Ser Ile Ala Gly
<210> SEQ ID NO 274
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
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Thr Pro Glu Gln Asn Val Glu Leu Ala Ala Ala Glu Leu Lys Ala Asp
Glu Lys Ser His Ala Val Ile Leu Gly Asp Thr Arg Tyr Gly Ser Glu
Glu Lys Gly Thr Tyr His Leu Ala Leu Phe Gly Asp Arg Ala Gln Glu
                          40
Ile Ala Gly Ser Ala Thr Val Lys Ile Arg Glu Lys Val His Glu Ile
Gly Ile Ala Gly
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<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: fHbp sequence variant
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Thr Pro Glu Gln Asn Val Glu Leu Ala Ser Ala Glu Leu Lys Ala Asp
Glu Lys Ser His Ala Val Ile Leu Gly Asp Thr Arg Tyr Gly Gly Glu
Glu Lys Gly Thr Tyr His Leu Ala Leu Phe Gly Asp Arg Ala Gln Glu
Ile Ala Gly Ser Ala Thr Val Lys Ile Gly Glu Lys Val His Glu Ile
Gly Ile Ala Gly
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<210> SEQ ID NO 276
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
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Glu Lys Ser His Ala Val Ile Leu Gly Asp Thr Arg Tyr Gly Ser Glu
                              25
Glu Lys Asp Thr Tyr His Leu Ala Leu Phe Gly Asp Arg Ala Gln Glu
Ile Ala Gly Ser Ala Thr Val Lys Ile Arg Glu Lys Val His Glu Ile
Gly Ile Ala Gly
65
<210> SEQ ID NO 277
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223 > OTHER INFORMATION: fHbp sequence variant
<400> SEQUENCE: 277
Thr Pro Glu Leu Asn Val Asp Leu Ala Ser Ala Glu Leu Lys Ala Asp
1 5
                          10
Glu Lys Ser His Ala Val Ile Leu Gly Asp Thr Arg Tyr Gly Glu
Glu Lys Gly Thr Tyr His Leu Ala Leu Phe Gly Asp Arg Ala Gln Glu
                         40
Ile Ala Gly Ser Ala Thr Val Lys Ile Arg Glu Lys Val His Glu Ile
   50
                      55
Gly Ile Ala Gly
65
<210> SEQ ID NO 278
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<400> SEQUENCE: 278
Ala Gln Gly Ala Glu
<210> SEQ ID NO 279
<211> LENGTH: 5
<212> TYPE: PRT
<213 > ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<400> SEQUENCE: 279
Ser His Phe Asp Phe
<210> SEQ ID NO 280
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223 > OTHER INFORMATION: Synthetic peptide
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<400> SEQUENCE: 280 His Leu Ala Leu

What is claimed is:

1. A non-naturally occurring factor H binding protein (fHbp), comprising a non-naturally occurring amino acid sequence, contiguously from its N-terminus to C-terminus:

$$\mathbf{V}_{A}\alpha\textbf{-}\mathbf{I}_{3}\textbf{-}\mathbf{V}_{B}\alpha\textbf{-}\mathbf{I}_{4}\textbf{-}\mathbf{V}_{C}\alpha\textbf{-}\mathbf{I}_{5}\textbf{-}\mathbf{V}_{D}\beta\textbf{-}\mathbf{I}_{6}\textbf{-}\mathbf{V}_{E}\beta$$

wherein the  $V_{A\alpha}$ ,  $V_{B\alpha}$ ,  $V_{C\alpha}$ ,  $V_{D\beta}$ , and  $V_{E\beta}$  are variable

wherein combination of alleles for each of the variable segments is not found in nature, and wherein:

the  $V_{A\alpha}$  has an amino acid sequence at least 85% identical  $_{20}$ to SEQ ID NO: 15,

the I<sub>3</sub> is the amino acid sequence SRFDF (SEQ ID NO: 3), the  $V_{B\alpha}$  has an amino acid sequence at least 90% identical to SEQ ID NO: 85,

the  $I_4$  is the amino acid sequence GEFQ (SEQ ID NO: 4), the  $V_{C\alpha}$  has an amino acid sequence at least 85% identical to SEQ ID NO: 96,

the  $I_5$  is the amino acid sequence DD,

the  $V_{DB}$  has an amino acid sequence at least 85% identical to SEQ ID NO: 192,

the I<sub>6</sub> is the amino acid sequence IEHLK (SEQ ID NO: 5) or IEHLE (SEQ ID NO: 6), and

the  $V_{E\beta}$  has an amino acid sequence at least 85% identical to SEQ ID NO: 262.

2. The non-naturally occurring fHbp of claim 1, wherein 35 said non-naturally occurring fHbp comprises, contiguously from N-terminus to C-terminus:

$$I_1$$
-Nte- $I_2$ - $V_A\alpha$ - $I_3$ - $V_B\alpha$ - $I_4$ - $V_C\alpha$ - $I_5$ - $V_D\beta$ - $I_6$ - $V_E\beta$ - $I_7$ ,

wherein the I<sub>1</sub> is CSSG (SEQ ID NO: 2),

wherein the Nte is a N-terminal element having the sequence G, GG, SGG, GGGS (SEQ ID NO: 7), SGSGG (SEQ ID NO: 8), GGGSGG (SEQ ID NO:9), GGGSGS (SEQ ID NO:10), GSGG (SEQ ID NO:11), GGGSGGGG (SEQ ID NO:12), GGGSGGGSGG 45 (SEQ ID NO:13), or GGSGG (SEQ ID NO:14),

the I<sub>2</sub> is GG, and

the  $I_7$  is the amino acid sequence KQ.

- 3. An immunogenic composition comprising a first nonnaturally occurring fHbp, wherein the first non-naturally 50 occurring fHbp is the non-naturally occurring fHbp of claim 1, and a pharmaceutically acceptable excipient.
- 4. The immunogenic composition of claim 3, further comprising a second N. meningitidis fHbp, wherein said second fHbp is different from said first non-naturally occurring fHbp. 55
- 5. The immunogenic composition of claim 3, wherein said non-naturally occurring fHbp is in a vesicle preparation, wherein vesicles are prepared from a *Neisseria* bacterium.
- 6. The immunogenic composition of claim 5, wherein said Neisseria bacterium is genetically modified to disrupt pro- 60 duction of an endogenous fHbp polypeptide.
- 7. The immunogenic composition of claim 3, wherein said non-naturally occurring fHbp is produced by a Neisseria bacterium that is genetically modified to produce said fHbp.
- 8. The immunogenic composition of claim 6, wherein said vesicle preparation is from the Neisseria bacterium producing said non-naturally occurring fHbp.

9. The immunogenic composition of claim 6, wherein said Neisseria bacterium is deficient in capsular polysaccharide synthesis.

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- 10. The non-naturally occurring fHbp of claim 1, wherein the V<sub>4G</sub> has an amino acid sequence at least 90% identical to SEQ ID NO: 15,
- the  $V_{Ba}$  has an amino acid sequence at least 90% identical to SEQ ID NO: 85,
- the  $V_{Ca}$  has an amino acid sequence at least 90% identical to SEQ ID NO: 96,
- the  $V_{DB}$  has an amino acid sequence at least 90% identical to SEQ ID NO: 192, and
- the  $V_{EB}$  has an amino acid sequence at least 90% identical to SEQ ID NO: 262.
- 11. The non-naturally occurring fHbp of claim 1, wherein the  $V_{A\alpha}$  has an amino acid sequence at least 95% identical to SEQ ID NO: 15,
- the  $V_{{\it B}\alpha}$  has an amino acid sequence at least 90% identical to SEQ ID NO: 85,
- the  $V_{C\alpha}$  has an amino acid sequence at least 95% identical to SEQ ID NO: 96,
- the  $V_{DB}$  has an amino acid sequence at least 90% identical to SEQ ID NO: 192, and
- the  $V_{E\beta}$  has an amino acid sequence at least 95% identical to SEQ ID NO: 262.
- 12. The non-naturally occurring fHbp of claim 2, wherein the  $V_{A\alpha}$  has an amino acid sequence at least 90% identical to SEQ ID NO: 15,
- the  $V_{B\alpha}$  has an amino acid sequence at least 90% identical to SEQ ID NO: 85,
- the  $V_{C\alpha}$  has an amino acid sequence at least 90% identical to SEQ ID NO: 96,
- the  $V_{\mathcal{D} \mathbf{B}}$  has an amino acid sequence at least 90% identical to SEQ ID NO: 192, and
- the  $V_{EB}$  has an amino acid sequence at least 90% identical to SEQ ID NO: 262.
- 13. The non-naturally occurring fHbp of claim 2, wherein the  $V_{A\alpha}$  has an amino acid sequence at least 95% identical to SEQ ID NO: 15,
- the  $V_{B\alpha}$  has an amino acid sequence at least 90% identical to SEQ ID NO: 85,
- the  $V_{C\alpha}$  has an amino acid sequence at least 95% identical to SEQ ID NO: 96,
- the  $V_{{\cal D}\beta}$  has an amino acid sequence at least 90% identical to SEQ ID NO: 192, and
- the  $V_{E\beta}$  has an amino acid sequence at least 95% identical to SEQ ID NO: 262.
- 14. The non-naturally occurring fHbp of claim 1, wherein said fHbp comprises an epitope that is specifically bound by JAR 5 monoclonal antibody.
- 15. A method of eliciting an antibody response to Neisseria meningitidis in a mammal, the method comprising administering to said mammal a composition comprising an immunologically effective amount of the non-naturally occurring fHbp of claim 1.
- 16. The method of claim 15, wherein the mammal is a human.

17. The method of claim 15, wherein said non-naturally occurring fHbp is in a preparation comprising outer membrane vesicles, microvesicles, or a mixture thereof.

**18**. The method of claim **17**, wherein said vesicles are prepared from two or more different strains of *N. meningiti-* 5 *dis*, each of which is genetically different from the other.

\* \* \* \* \*